A.M.A.

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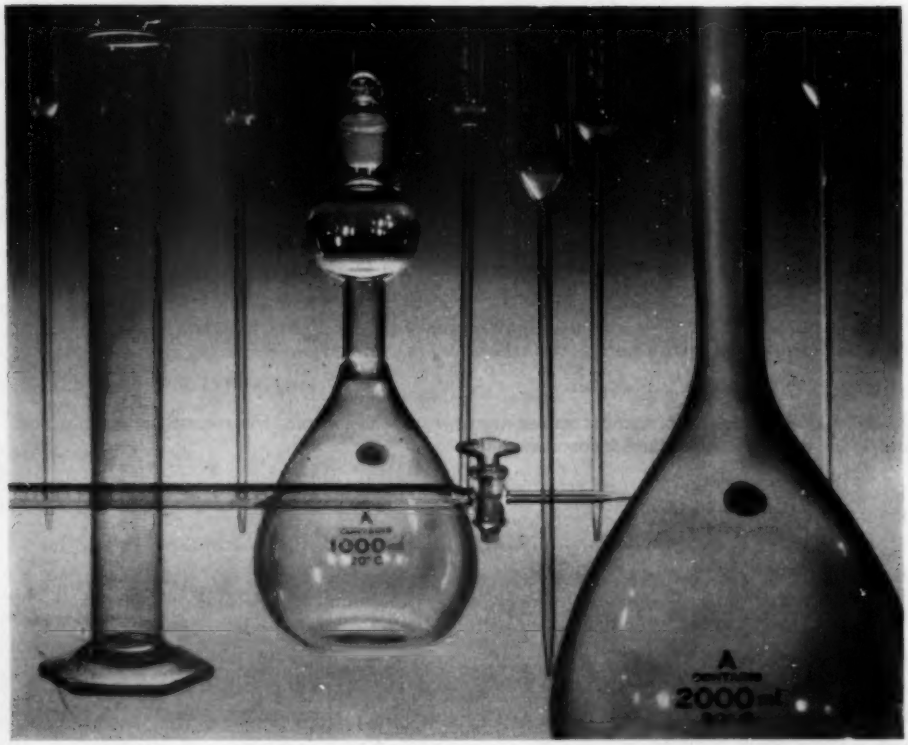

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A.M.A. Archives OF PATHOLOGY

Editorials

This issue marks the formal announcement of an affiliation between the A. M. A. Archives of Pathology and the American Society for Experimental Pathology. The members of this important organization of pathologists, after careful deliberation, voted to accept the offer of affiliation extended to them by the A. M. A. Archives of Pathology. They did so, presumably, because of their belief that experimental pathology had attained a stature warranting a more definite recognition in the general field of pathology. Rather than deciding to establish a journal of their own or to affiliate with a specialized type of journal, many of them preferred to join forces with a journal already devoted to the publication of diversified types of papers in the broad field of pathology.

In the preliminary negotiations, representatives of the American Medical Association evidenced a hearty interest in the objectives of the members of the American Society for Experimental Pathology. A primary purpose of the American Medical Association has been to publish specialty journals of optimal usefulness and of widening interest to their readers; in fact, those responsible for the production of these journals are themselves "experimentalists" in that they are constantly striving to improve the Specialty Journals in various ways.

The affiliation does not signify any drastic change of policy for the A. M. A. Archives of Pathology, and there will be a continued effort to encourage the publication of a diversified type of material. It does mean, however, that good papers predominantly experimental in nature will be cordially welcomed, as, indeed, they have been in the past. It is to be hoped, moreover, that the affiliation will ensure a closer feeling of sympathy on the part of pathologists of specialized interests with the broad objectives of the American Medical Association and its Specialty Journals.

As Chief Editor of the A. M. A. Archives of Pathology, I wish to assure the members of the American Society for Experimental Pathology that we of the Archives shall continue to have as a primary objective the publishing of a journal which will be of growing interest and of increasing scientific and practical value. We hope, too, that our efforts will also help to promote a widening interest in all aspects of pathology, thereby adding to the growth of medicine itself.

Paul R. Cannon

Precipitation of Sulfadiazine in the Heart

JACOB CHURG, M.D.
and
BLANKA SENDER, M.D., Paterson, N. J.

Precipitation of poorly soluble sulfonamides in the kidneys is a well-known phenomenon. It occurs when the concentration of the drug exceeds the carrying capacity of the urine and is promoted by conversion of the sulfonamide to a less soluble (e. g., acetylated) form. Similarly, a sulfonamide which is maintained in solution in the form of a sodium salt at a high pH may be thrown down when neutralized in the body, e. g., in the peritoneal cavity of experimental animals. Under unusual circumstances sulfonamide can precipitate out from the blood stream, as illustrated by the following case.

Submitted for publication May 2, 1956.
From the Laboratories, Barnert Memorial Hospital.

Report of a Case

A 30-year-old Negro man was admitted to the Barnert Memorial Hospital in deep coma. The history obtained from the family indicated progressive loss of weight, unusual thirst, and increasing weakness of several months' duration. The patient had never consulted a physician until the day before admission. Sugar and acetone in the urine, high blood sugar (674 mg. per 100 cc.), and low carbon-dioxide-combining power (16 mEq.) established the diagnosis of diabetic coma. Energetic treatment with insulin and intravenous fructose and corticotropin was instituted, but without any benefit to the patient. The temperature, which was 102 F on admission, rapidly rose to 107.8 F. The blood pressure dropped to 55/40 mm. Hg. Because of the strong suspicion of an overwhelming infection, such as meningococcemia, 7.5 gm. of sulfadiazine sodium was given in an intravenous drip of N/6 molar sodium lactate over a period of 30 minutes. The patient died about 20 minutes after conclusion of the injection and 6 hours after admission to the hospital.

Fig. 1.—Right ventricle of the heart, showing granules of sulfadiazine precipitate covering the mural endocardium.

At autopsy, performed one hour after death, significant changes were found in two organs: The pancreas showed diffuse serous and purulent

inflammation, with fat necrosis; this was superimposed upon focal, mild to moderate lobular fibrosis. The heart appeared dilated and soft, but not hypertrophied. It weighed 360 gm. Upon opening of the right auricle and ventricle numerous minute white granules were seen attached to the mural endocardium and to the tricuspid valve (Figs. 1 and 2). The granules had the appearance of small burrs, measuring about 1 mm. in diameter. They could be removed only with difficulty by scraping with a sharp knife. Mounted in balsam and examined under the microscope, they were yellowish and shaped like scalloped globules or conglomerations of globules. Under reduced light, they showed fine radial crystalline structure, the crystals being doubly refractile when examined with polarized light. Upon treatment with Ehrlich's *p*-dimethylaminobenzaldehyde reagent, the granules were colored bright yellow; with the Bratton-Marshall diazo procedure they turned purple-red. No granules were found in the pulmonary arteries or veins, or anywhere in the left heart. The kidneys were also free of precipitate.

Fig. 2.—Same as Figure 1. Close-up of trabeculae carneae.

Microscopic sections of the right auricle and ventricle showed the granules attached to the endocardium (Fig. 3), or sometimes lying free within small blood clots. The individual globules measured 20μ to 250μ, most frequently 50μ to 100μ. The fine radial architecture was clearly visible (Fig. 4), but double refractility and color reactions with the Ehrlich and Bratton-Marshall reagents were no longer present. The granules stained red with eosin, pale orange with phosphotungstic-acid hematoxylin, and predominantly red with the chromotrope-aniline blue modification of Mallory's connective tissue stain. These reactions indicate the presence of protein, presumably precipitated plasma proteins. The free surface of many of the globules was covered by flattened leucocytes, and a few cells were seen also inside the granules. In addition to the chambers of the right heart, the

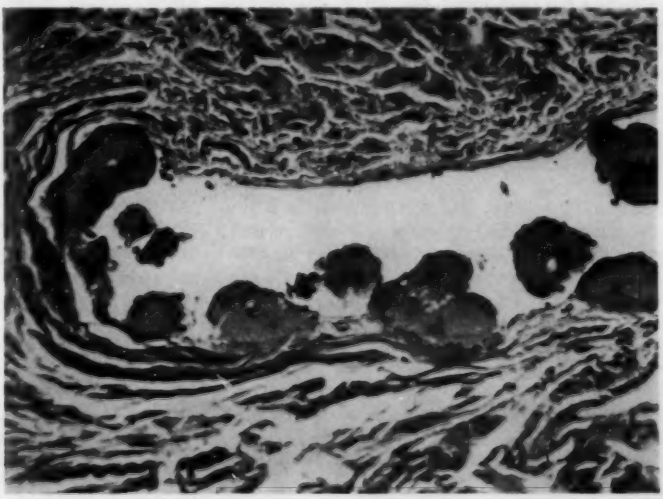

Fig. 3.—Right ventricle at the point of attachment of the tricuspid valve. Sulfadiazine "globules" adhere to the valve and to the mural endocardium. × 135.

Fig. 4.—Two sulfadiazine "globules" showing distinct radial structure. × 135.

granules were found in small coronary veins, attached to the intima. None were seen in the coronary arteries or in numerous sections of the lungs.

Comment

The cause of death was irreversible diabetic coma precipitated or aggravated by acute pancreatitis. Though the dose of sulfadiazine was fairly high, it did not exceed the limits (0.075-0.1 gm. per kilogram of body weight) recommended in severe infection, and it was administered over a period of 30 minutes. One of the main factors conducive to precipitation of sulfadiazine from the blood stream was undoubtedly a circulatory stasis induced by the terminal shock, and resulting in a high local concentration of the drug in the right heart. Another possible factor was acidemia, leading to more rapid dissociation of sodium from the sulfadiazine molecule.

The precipitate assumed the shape of scalloped globules attached to the endocardium and consisted of needle-like, doubly refractile crystals of sulfadiazine arranged radially and held together by a coprecipitate of plasma proteins. The globules were generally similar to the typical crystals of sulfadiazine observed in the urine of patients receiving the drug.[1] In the paraffin section, the protein matrix of the globules was preserved, but the drug was removed in the process of dehydration of tissues by alcohol.

Summary

Extensive precipitation of sulfadiazine was observed in the right cardiac auricle and ventricle and in the small coronary veins of a patient who received the drug intravenously shortly before death.

REFERENCE

1. Kolmer, J. A.; Spaulding, E. H., and Robinson, H. W.: Approved Laboratory Technic, Ed. 5, New York, Appleton-Century-Crofts, Inc., 1951, p. 183.

Calcium Concentrations in Sclerotic Cerebral Arteries

J. C. PATERSON, M.D.
and
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Structurally, the cerebral arteries differ considerably from other musculoelastic arteries of similar size.[1] The external elastic lamina is absent and the adventitia is poorly developed; the musculature is thinner than in other arteries and contains only a few delicate elastic fibrils; on the other hand, the amount of elastic tissue in the internal elastic lamina is extremely abundant, making this lamina unusually thick. All of these differences suggest that the greatest strength of the vessel lies in its inner layers. As Benninghoff points out,* they may be an adaptation to the absence of any need for protection against external stresses, in addition to satisfying the obvious requirement of resisting the blood pressure within the lumen. A further difference has recently been reported by Buck and associates,[2] one that may also influence the strength of cerebral arteries: They found unusually low concentrations of calcium in cerebral arteries even when severe grades of atherosclerosis were present.

In the course of a recent survey, part of which has been reported in detail elsewhere,† we have been able to confirm this latter observation. Although the concentration of lipid in sclerotic cerebral arteries in our series was in the same general range as that in coronary arteries with similar grades of disease, the concentration of calcium in these cerebral arteries was much lower.

Submitted for publication June 6, 1956.

From the Clinical Investigation Unit of Westminster Hospital, Department of Veterans Affairs, and the Collip Medical Research Laboratory, University of Western Ontario.

* Benninghoff, A., cited by Duff.[1]

† References 3 and 4.

Material and Method

The material was obtained from the first 71 fatalities in a series of 800 patients who are permanently confined to hospital and on whom serum lipid levels are being estimated at least once a year during life.† At autopsy the grade of atherosclerosis in the coronary, cerebral, and femoral arteries and in the abdominal aorta was estimated by a number of indices, including the concentration of lipid and of calcium in each type of vessel. The estimations in the coronary and cerebral arteries were carried out as follows:

The entire epicardial portions of the coronary arteries were excised, opened carefully with scissors, and stripped manually of their outer medial and adventitial coats to remove contaminating fat. The morphological grade of atherosclerosis was determined, using the criteria of Davis and Klainer,[3] by which the degree of stenosis produced by the largest plaque is the critical feature. When atherosclerotic plaques were infrequent and did not encroach appreciably upon the lumen, the disease was graded as slight; when plaques were more numerous and protruded slightly but appreciably into the lumen, the disease was graded as moderate, and when one or more plaques stenosed the lumen to 50% or more of its usual diameter, the disease was graded as severe. A small segment of the largest plaque from the specimen was taken and studied histologically. The remainder of the material was submitted to the chemical laboratory for tissue analysis.

The cerebral arteries were handled in the same fashion, with one exception: Since the adventitia of these vessels does not contain fat, the stripping procedure used in the coronary arteries was not followed. The specimen included the basilar artery, the circle of Willis, and approximately ½ in. of all major branches, including the intracranial portion of the internal carotid artery and the vertebral arteries.

In the chemical laboratory the fresh tissues from the coronary and cerebral vessels were weighed at once and subjected to alkaline digestion and storage as recommended by Haven,

Bloor, and Randall.[6] After complete digestion an estimation of the total lipid content was made by the method of Haven, Bloor, and Randall[6]. Another aliquot of the alkaline digest was subjected to acid digestion by the method of Ma and Zuazaga,[7] and the calcium content of this digest was estimated by the Clarke-Collip[8] modification of the Kramer-Tisdall method. (By this procedure we were unable to measure accurately calcium in quantities less than 0.1 mg. per 100 cc.)

The morphological and chemical estimations were carried out independently by two groups of workers. Only when all of the data had been compiled were the two sets of estimations compared. The first step was to arrange the series into three major groups: One group was made up of cases with severe grades of coronary and cerebral atherosclerosis; another group, with moderate grades of disease, and a third, with slight grades. Then, the concentrations of lipid and of calcium in arteries in the three groups were compared statistically by the Fisher method.[9] *P* values of less than 0.05 were considered significant, while those equal to 0.05 were considered of borderline significance.

TABLE 1.—*Lipid and Calcium Concentrations in Coronary and Cerebral Arteries with Severe Atherosclerosis*

Coronary Arteries					Cerebral Arteries				
Case No.	Age, Yr.	Sequelae*	Lipid Conc., Mg/100 Cc.	Calcium Conc., Mg/100 Cc.	Case No.	Age, Yr.	Sequelae*	Lipid Conc., Mg/100 Cc.	Calcium Conc., Mg/100 Cc.
1	84	+	14.0	10.4	1	84	−	6.5	0.3
2	80	+	3.3	1.0	3	70	+	2.2	0.1
3	70	−	5.3	3.5	31	74	+	4.9	<0.1
4	92	+	7.1	1.8	4	92	−	5.2	0.1
5	74	−	4.6	8.7	5	74	+	3.1	<0.1
6	59	+	8.4	0.4	6	59	−	8.7	0.1
7	39	+	6.4	0.6	8	74	+	2.2	<0.1
8	74	−	6.0	1.5	12	64	+	2.1	0.2
9	70	+	8.0	2.1	32	81	+	2.5	0.3
10	57	+	4.0	2.3	33	69	−	3.1	0.1
11	62	+	1.5	4.0	34	62	+	7.0	1.3
12	64	+	0.8	7.9	35	84	−	4.2	<0.1
13	82	+	6.0	6.4	18	83	+	8.1	0.8
14	54	+	2.4	1.1	36	59	−	6.0	<0.1
15	63	+	3.1	3.3	19	88	−	5.5	0.6
16	67	−	6.2	5.6	37	71	+	3.6	<0.1
17	74	−	5.9	6.6	20	81	+	3.8	0.1
18	83	+	5.5	2.1	38	77	−	8.7	<0.1
19	88	+	4.2	2.7	39	72	+	4.2	<0.1
20	81	−	7.5	5.6	25	76	−	3.3	<0.1
21	57	+	7.8	2.9	29	87	+	6.0	<0.1
22	89	+	5.3	0.9					
23	67	−	5.2	4.2					
24	76	+	5.6	2.1					
25	76	+	5.8	1.5					
26	62	−	5.8	<0.1					
27	72	−	5.5	2.5					
28	71	−	3.5	5.7					
29	87	−	10.9	<0.1					
30	77	−	6.3	2.2					

* The sequelae of coronary atherosclerosis were taken to be a coronary thrombus, a massive occluding intimal hematoma, a cardiac infarct (old or recent), or sudden and unexpected death in association with severe coronary sclerosis and no other obvious cause for sudden death. Of cerebral atherosclerosis, they were a cerebral artery thrombus or occluding hematoma, a cerebral hemorrhage, or a cerebral infarct.

TABLE 2.—*Lipid and Calcium Concentrations in Coronary and Cerebral Arteries with Moderate Atherosclerosis*

Coronary Arteries					Cerebral Arteries				
Case No.	Age, Yr.	Sequelae*	Lipid Conc., Mg/100 Cc.	Calcium Conc., Mg/100 Cc.	Case No.	Age, Yr.	Sequelae*	Lipid Conc., Mg/100 Cc.	Calcium Conc., Mg/100 Cc.
1	64	−	2.8	0.5	30	74	−	2.2	<0.1
2	84	+	5.3	0.3	1	64	−	3.6	<0.1
3	73	−	3.3	0.1	2	84	−	1.3	<0.1
4	59	−	3.2	0.5	4	59	−	1.6	<0.1
5	74	−	1.4	0.8	31	70	−	0.7	1.7
6	65	−	3.0	0.4	5	74	−	0.6	0.9
7	81	−	4.0	5.0	32	62	−	0.8	<0.1
8	69	−	4.5	4.2	33	82	−	3.0	0.5
9	56	−	2.1	0.2	34	54	−	2.1	0.1
10	62	−	5.2	1.2	11	68	−	1.9	<0.1
11	68	−	5.2	2.0	12	82	+	1.9	0.2
12	82	−	3.5	1.2	35	74	−	1.1	<0.1
13	70	−	7.4	4.1	13	79	+	1.4	<0.1
14	61	−	5.2	4.4	36	57	−	4.2	<0.1
15	84	+	4.8	4.9	21	84	+	2.8	<0.1
16	59	−	4.6	0.1	22	74	+	11.4	<0.1
17	71	−	3.7	0.8	37	67	−	5.5	<0.1
18	79	−	6.4	1.6	38	76	−	6.9	<0.1
19	49	−	3.4	<0.1	23	85	−	8.4	<0.1
20	77	−	4.6	2.7	39	62	−	3.8	<0.1
21	84	−	5.1	1.7	40	72	−	4.5	<0.1
22	74	−	3.3	<0.1	41	71	−	4.9	<0.1
23	85	−	3.3	1.2	42	77	+	5.5	<0.1
24	72	+	4.4	<0.1	29	76	−	4.1	<0.1
25	58	−	4.4	<0.1	43	78	+	7.6	<0.1
26	62	−	2.9	<0.1	44	76	−	4.7	<0.1
27	62	−	6.1	0.7					
28	46	−	5.0	<0.1					
29	76	−	6.2	0.4					

* The sequelae of coronary atherosclerosis were taken to be a coronary thrombus, a massive occluding intimal hematoma, or a cardiac infarct (old or recent). Of cerebral atherosclerosis, they were a cerebral artery thrombus or occluding hematoma, a cerebral hemorrhage, or a cerebral infarct.

Observations

Even on gross examination it was obvious that there was a marked difference in the amount of calcification in coronary and cerebral arteries which showed similar morphological grades of disease. Severely affected coronary arteries were often so hard that they could not be cut with the scalpel without fracturing their walls. Severely sclerotic cerebral arteries, on the other hand, could always be cut easily and cleanly with the knife. Histological study of plaques in the two types of arteries supported the impression obtained from gross examination. Deposits of granular material, interpreted as calcium, were seen in most of the plaques in the coronary arteries (in 60 out of 71 cases), but they were infrequent in plaques in the cerebral vessels (in 12 out of 70 cases).

The results of our chemical analyses showed conclusively that these morpholog-

TABLE 3.—*Lipid and Calcium Concentrations in Coronary and Cerebral Arteries with Slight Atherosclerosis*

Coronary Arteries					Cerebral Arteries				
Case No.	Age, Yr.	Sequelae*	Lipid Conc., Mg/100 Cc.	Calcium Conc., Mg/100 Cc.	Case No.	Age, Yr.	Sequelae*	Lipid Conc., Mg/100 Cc.	Calcium Conc., Mg/100 Cc.
1	55	+	4.7	1.9	1	55	−	2.0	<0.1
2	74	+	4.7	0.1	3	74	−	3.5	<0.1
3	74	−	3.8	<0.1	13	80	−	2.4	<0.1
4	74	−	4.4	0.2	5	54	−	4.4	<0.1
5	54	−	4.5	0.4	14	71	−	2.0	0.1
6	59	−	3.0	1.0	6	59	−	2.1	0.1
7	59	−	4.8	<0.1	7	59	−	2.7	<0.1
8	83	−	0.7	<0.1	15	57	−	1.9	<0.1
9	33	−	2.1	0.2	8	83	−	0.7	<0.1
10	66	−	3.6	<0.1	16	65	−	1.1	<0.1
11	78	−	4.8	1.1	17	56	−	0.9	<0.1
12	76	−	4.9	<0.1	18	63	−	1.8	<0.1
					19	67	−	0.5	<0.1
					20	61	+	1.2	<0.1
					21	89	−	1.4	<0.1
					22	79	−	3.5	<0.1
					23	49	−	3.4	<0.1
					24	58	−	2.0	<0.1
					25	62	−	1.7	<0.1
					26	62	−	3.8	<0.1
					27	46	−	4.8	<0.1
					9	33	−	3.7	<0.1
					10	66	−	5.0	<0.1

* The sequelae of coronary atherosclerosis were taken to be a coronary thrombus, a massive occluding intimal hematoma, or a cardiac infarct (old or recent). Of cerebral atherosclerosis, they were a cerebral artery thrombus or occluding hematoma, a cerebral hemorrhage, or a cerebral infarct.

ical impressions were correct. The concentrations of lipid and calcium in the coronary and cerebral arteries of each of the 71 cases are given in Tables 1, 2, and 3. Table 1 shows the concentrations in arteries graded morphologically as severely atherosclerotic; Table 2, the concentrations in arteries with moderate atherosclerosis, and Table 3, those in arteries with slight grades of disease. It will be seen in Table 1 that, with only a few exceptions, the concentration of calcium in severely sclerotic cerebral arteries was negligible. In almost half of the cases it was so low that it could not be measured accurately by our method. On the other hand, appreciable concentrations of the mineral were found in almost every specimen of coronary arteries affected by a severe grade of disease. Tables 2 and 3, similarly, show considerably lower levels of calcium concentration in the cerebral arteries than in the corresponding coronary arteries.

Casual examination of these three Tables reveals two other points of interest. The levels of lipid concentration in coronary and cerebral arteries with the same morphological grade of disease were similar, and the incidence of the sequelae of atherosclerosis was approximately the same in organs supplied by the two arterial systems.

The data given in Tables 1, 2, and 3 have

TABLE 4.—*Lipid Concentration of Coronary and Cerebral Arteries with Different Morphological Grades of Atherosclerosis*

Grade	Lipid, Mg/100 Cc.			
	Severe	Moderate	Slight	Severe cf. Slight
Coronary	5.7±0.5*(30)†	4.3±0.3 (29)	3.8±0.4 (12)	P=0.02
Cerebral	4.8±0.5 (21)	3.7±0.5 (26)	2.5±0.3 (23)	P<0.001
Coronary cf. cerebral	P>0.10	P>0.10	P<0.01	

* Mean±standard error of the mean.
† Number of cases.

TABLE 5.—*Calcium Concentration of Coronary and Cerebral Arteries with Different Morphological Grades of Atherosclerosis*

Grade	Calcium, Mg/100 Cc.*			
	Severe	Moderate	Slight	Severe cf. Slight
Coronary	3.3±0.5† (30)‡	1.4±0.3 (29)	0.5±0.2 (12)	P<0.001
Cerebral	0.2±0.1 (21)	0.2±0.1 (26)	0.1±0.0 (23)	P>0.10
Coronary cf. cerebral	P<0.001	P=0.001	P<0.01	

* In order to calculate this mean value, all values less than 0.1 mg. per 100 cc. were considered to be 0.1 mg. per 100 cc.
† Mean±standard error of the mean.
‡ Number of cases.

TABLE 6.—*Age Distribution of the Cases Comprising This Series*

Grade	Severe	Moderate	Slight	Severe Cf. Slight
Coronary	72±2* (30)†	69±2 (29)	65±4 (12)	P>0.10
Cerebral	75±2 (21)	72±2 (26)	63±3 (23)	P=0.001
Coronary cf. cerebral	P>0.10	P>0.10	P>0.10	

* Mean age±standard error of the mean.
† Number of cases.

been summarized and treated statistically. The results are given in Tables 4 and 5. It is seen that (1) there was a significant increase in lipid concentration in both coronary and cerebral arteries with increased severity of atherosclerosis (Table 4); (2) whereas the calcium concentration of sclerotic coronary arteries increased significantly with increased severity of disease, there was no significant difference in the calcium concentration of cerebral arteries with severe and with slight grades of disease (Table 5), and (3) although coronary and cerebral arteries of the same morphological grade of sclerosis were found to have similar levels of lipid concentration, the concentration of calcium in cerebral arteries was markedly, and significantly, lower than that of coronary arteries with the same grade of disease.

Finally, the data on the age distribution of cases in the series have been summarized and are presented in Table 6. It will be noted that the mean age of cases with differing degrees of coronary sclerosis was not significantly different from that of cases with the same degrees of cerebral sclerosis, and that age appeared to be a factor in the progression of cerebral atherosclerosis (but not of coronary atherosclerosis) as determined by gross morphological grading.

Comment

The observations reported in this paper confirm those of Buck and associates that calcium deposition is extraordinarily slight in atherosclerotic cerebral arteries.[2] Exceptional cases were encountered in which the cerebral arteries did contain appreciable amounts of this mineral, but these resulted, we believe, from the fact that the distal end of the internal carotid artery was always included in our specimen. Buck and associates did not use any part of this vessel in their studies.[2] They did, however, show that the interosseous portion of the internal carotid artery is prone to calcification.

The reason for the failure of atherosclerotic plaques of the cerebral arteries to become impregnated with calcium was not apparent in our studies. Nevertheless, the implications of this failure are of some interest. It means, first, that the amount of calcification in this type of vessel cannot be used as an index of the severity of atherosclerosis. This may throw doubt on the validity of using calcification as an index of the severity of the disease in any artery. It probably means also that calcium deposition, in cerebral arteries at least, is not an important factor in the progression of the disease process. Although cerebral artery thrombosis, cerebral hemorrhage, and cerebral infarction were quite common in association with severe cerebral atherosclerosis in our series, the arteries in these cases rarely contained appreciable amounts of calcium. Calcification is therefore not a prerequisite for vascular insufficiency in these vessels.

Finally, defective calcification may explain why atherosclerotic cerebral arteries are more prone to rupture than are other arteries similarly affected. Buck and associates have already made this suggestion, quoting the statement of Wells that "the infiltration of lime salts into diseased arteries may serve as a protective mechanism to prevent rupture."[2] No such protective device seems to exist in the major cerebral arteries, except perhaps in the intracranial stump of the internal carotid.

Summary and Conclusions

Chemical analyses of sclerotic coronary and cerebral arteries of 71 consecutive fatalities have shown that the concentration of calcium in the cerebral arteries is much lower than that in coronary arteries affected by atherosclerosis of similar degrees and with similar amounts of lipid deposited in their walls. The suggestion that defective calcification of sclerotic cerebral arteries may explain their tendency to rupture is supported.

Prof. R. J. Rossiter, DVA Consultant in Biochemistry, and Dr. J. B. Derrick gave advice and criticism in the course of this investigation. Dr. E. C. Armstrong and various members of the Westminster Hospital Staff—Miss Dorothy Marsh, Mr. J. C. D. Gilbert, Mr. T. Moffatt, and Mrs. Grace Strickland—also assisted.

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Studies of Four Strains of the Common Cold Virus in Suckling Hamsters

A Review

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The common cold (acute coryza, acute rhinitis) has been attributed to infection of the upper respiratory tract with a virus. The symptoms and signs indicate the presence of an acute but transient alteration in the physiology of the mucous membrane of the upper respiratory tract, particularly that lining the nose and paranasal sinuses. Dochez and associates,[1] first reported successful transmission of the common cold to chimpanzees by means of filtrates. Many research workers,* ourselves included, have attempted to introduce the cold virus into a number of mammals, with negative results, until our trials with suckling hamsters. Suckling hamsters can be obtained easily and economically. All suckling hamsters and mothers have been obtained from a reliable dealer in Silver Spring, Md., who stated that his hamster colony was in excellent condition.

Materials and Methods

CASE 1 (Strain MR): Saline washings from the nose and throat were obtained from a young woman two days after the onset of a typical common cold. The clinical picture was typical nasopharyngitis with a slight soreness or roughness of the throat. The nasal and nasopharyngeal mucosae were swollen and injected. The patient's temperature remained normal.[4]

CASE 2 (Strain C): Nasal and throat washings were obtained during the first two days of a cold from a worker in our laboratory. The clinical picture was as follows: Slight sneezing in the early morning followed by copious watery nasal discharge and by watering of the eyes during the day. In the late afternoon, he experienced slight headache and chilly sensations. The following day, he felt malaise with cessation of the nasal discharge during the afternoon. During the course of the cold, he never had cough, purulent nasal discharge, occluded nostrils, or elevated temperature.[5]

CASE 3 (Strain RLR): Nasal and throat washings were obtained from a patient during the first two days of a cold. The clinical picture was similar to that in Case 2 except that this patient had more sneezing and the cold was a little longer in duration (seven days).

CASE 4 (Strain D): Nasal and throat washings were obtained 14 hours after the onset of a common cold from a physician at Walter Reed Army Medical Center. He had had simple colds approximately twice a year for many years. He had no known allergies. One evening he recognized the prodomals of one of his usual colds. His head felt full, and he had a slight headache. The next morning he had typical symptoms of a common cold. Examination showed a temperature of 100 F, mild conjunctival infection, pharyngeal redness, and mild edema and nasal congestion. Nasal smears showed no eosinophiles. The cold ran a course of six days and was not complicated by significant secondary infections. None of the above patients had known allergies but were typical cases of the common cold.

Three lactating hamsters and their sucklings were placed in individual metal boxes with bedding. The sucklings were six days old. The sucklings from Groups 1 and 2 were given 0.03 ml. of the filtered specimen (Case 1) by nasal instillation. The sucklings from Group 3 were exposed in the same manner with filtered nose and throat

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* References 2-6.

washings from a normal patient. (This patient had had no signs of a common cold for the past year.) Dog Checkers were fed to each of the three groups daily, along with a large bottle of water for drinking. The same procedure as that in Case 1 was carried out for each of the other three cases of the cold virus. Results are given in the Table.

Cold Virus: Passage in Suckling Hamsters

Group No.	Strain Cold Virus	No. Showing Symptoms	No. Showing No Symptoms	Onset of Disease, Days
1,2	MR*	8/11	3/11	4
3 (control)	----	0	12/12	0
1,2	C	8/12	4/12	3-7
3 (control)	----	0	6/6	0
1,2	RLR	8/10	2/10	3-7
3 (control)	----	0	5/5	0
1,2	D	7/14	7/14	3-7
3 (control)	----	0	6/6	0

* Strain MR was passed serially in suckling hamsters via the intranasal route up to four passages. All other strains were carried only one passage.

After three to seven days, several sucklings in each group (except the controls) exhibited cold virus signs, such as running nose and wheezing, with the nostrils swollen, boggy and inflamed (Table). All controls remained normal throughout the experiment.

The fourth hamster passage of Strain MR and virus material from each of the other three strains were instilled nasally into suckling hamsters having recovered from the cold virus and into suckling having no cold symptoms. The immune animals exhibited no symptoms, while the nonimmune sucklings developed typical cold symptoms after three to seven days.

Summary

From the data obtained in these studies it is apparent that these four cold virus strains were transmitted to suckling hamsters. It was found that Strain MR was transmissible from suckling to suckling for four serial passages. The suckling hamster has proved to be the ideal animal for experimentation with the cold virus because of economy and ease of handling. As far as can be determined from available data, the suckling hamster is the only laboratory animal except the chimpanzee to which a cold virus has been transmitted. Confirmation of the presence of each cold virus strain was made by nasal instillation using immune and nonimmune hamsters.

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Mucosal Inflammatory Spread in Diverticulitis and Ulcerative Colitis

A Comparative Study

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The sigmoid colon is the site of origin for both ulcerative colitis and diverticulitis in the majority of cases.

Ulcerative colitis, of every variety, becomes a serious and unmanageable condition when it spreads to involve large areas of the colon, and it seems true to say that the more rapid the spread the more serious the clinical condition (Lumb, Protheroe, and Ramsay). In those cases of ulcerative colitis which pursue a relatively benign course, easily controlled by medical measures, the disease remains in the distal sigmoid colon and upper rectum. Sometimes in such cases the condition is given a special name, such as nonspecific proctosigmoiditis (Brooke), although in our experience there are no specific morphological changes which seem to justify such a subdivision (Lumb and Protheroe [3]). Occasionally localized disease may occur elsewhere in the colon, and in these cases the term regional segmental colitis has been suggested (Neuman, Bargen, and Judd [4]).

Studies of the mucosal changes in ulcerative colitis (Lumb and Protheroe [3]) have shown the very incomplete epithelial regeneration which occurs when the inflammatory process becomes quiescent and also the tendency for the lesions to spread locally to involve considerable areas. In the majority of cases of diverticulitis there is a localized lesion. When a series of cases in our hospital was reviewed, however, it was found that some produced spreading mucosal lesions, a few of which eventually became indistinguishable from ulcerative colitis. It was decided therefore to investigate the mucosal changes in these cases in order to determine whether any demonstrable differences existed between the localized and the spreading type of the disease and also to compare these changes with the changes in ulcerative colitis.

Material

One hundred sixty-four cases in which a clinical diagnosis of diverticulitis had been made were available for study. Of these, 57 had required surgery, and the specimens from 30 men and 27 women were available. The average age of the patients was 59.9 years. In 53 cases the disease was typical, in 21 of which it was in an acute inflammatory phase at the time of operation and in 32 quiescent. The remaining four cases had been complicated by changes indistinguishable from those of ulcerative colitis (Table 1). In one of these the disease had been classified as diverticulitis on clinical grounds, but when the colon was viewed at operation a diagnosis of segmental colitis was thought to be more likely. The other three cases showed radiological evidence of diverticulitis, but subsequently the clinical diagnosis was changed to ulcerative colitis in view of the course of the disease and altered radiological appearances (Table 2). Fifty-one specimens were portions of the sigmoid colon, varying in length from 14 to 30 cm. Three included the upper two-thirds of the rectum, and three were total colectomy specimens. The indications for surgical resection of colon are shown in Table 3.

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TABLE 1.—*Cases of Diverticulitis Treated Surgically*

	No. of Cases	Mucosal Lesions	
		Localized to Diverticula	Spreading
Acute cases	21	16	5
Quiescent cases	32	31	1
Cases complicated by ulcerative colitis	4	0	4
Total	57	47	10

ing 5 there was evidence of spread to involve the adjacent mucous membrane (Table 1).

In all these cases there was an acute inflammation which led to ulceration of the mucous membrane lining the sac. This process may remain localized to a small segment or involve the whole diverticulum. The point of origin may be in any part of the sac. Spread normally occurs through the surrounding thin muscle strands to produce a subserosal inflammatory process,

TABLE 2.—*Clinical Details of Cases of Diverticulitis Complicated by Acute Ulcerative Colitis*

Case No.	Sex	Age at Onset	Symptomatology	Radiological Study	Duration of Symptoms Before Diagnosis of Ulcerative Colitis	Duration of Symptoms Before Surgical Intervention	Extent of Disease at Operation
1	F	60	Intermittent diarrhea and constipation; slight rectal bleeding; pain in L.I.F.	Diverticulitis of sigmoid colon	12 mo.	18 mo.	Total colitis
2	M	48	Intermittent diarrhea and constipation; slight rectal bleeding; pain in L.I.F.	Diverticulitis of sigmoid colon	6 yr.	7 yr.	Distal half of transverse, descending colon, and rectum
3	F	47	Intermittent diarrhea and constipation; pain in L.I.F.	Diverticulitis of sigmoid colon	3 yr.	3½ yr.	Total colitis
4	F	60	Constipation; abdominal pain; mass in L.I.F.; loss of weight; slight rectal bleeding	Diverticulitis of sigmoid colon	6 mo.	6 mo.	24 cm. of sigmoid colon and rectum

TABLE 3.—*Indications for Surgical Resection of Colon*

	No. of Cases
Obstruction due to fibrosis and angulation	21
Obstruction with carcinoma	3
Obstruction with suspected carcinoma; not confirmed at operation	6
Pericolic abscess	12
Vesicocolic fistula	3
Recurrent attacks of acute diverticulitis	8
Complication of acute ulcerative colitis	4
Total	57

Acute Cases

In 21 of the surgical specimens there was evidence of acute inflammation, which in 16 was confined to the diverticula and peridiverticular tissues, and in the remain-

sometimes associated with abscess formation (Fig. 1). Adjacent viscera may, in turn, be involved, with subsequent fistula formation. Abscesses are not found in all cases, and a characteristic appearance is a spreading nonsuppurative peritonitis with edema, thickening, and congestion of the mesentery and pericolic connective tissues. In such cases it may require careful dissection, with removal of the pericolic fat, to discover the affected diverticula.

The histological picture shows no specific characteristics and is that of a typical acute inflammatory process with superficial mucosal erosions and ulcers associated with polymorphonuclear leucocytic infiltration. There is some stromal edema, but eosinophilic infiltration, although sometimes seen, is not a feature of this condition. In the majority of cases the changes diminish in intensity at the mouth of the diverticula

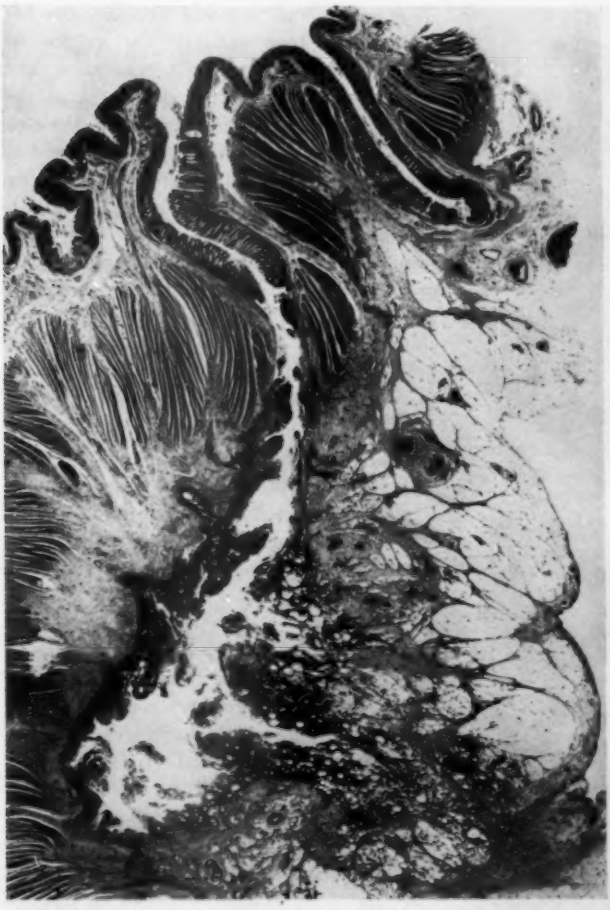

Fig. 1. — Ruptured diverticulum with subserosal abscess formation. Hematoxylin and eosin stain; reduced to 70% of mag. × 3.5.

and merge with the normal mucosa of the free surface (Fig. 1).

A surgical specimen removed during the acute phase of the disease will frequently show multiple diverticula most of which are normal adjacent to a group which are inflamed.

When viewed externally, the changes beneath the serosa tend to be out of all proportion to the extent of mucosal involvement. It would appear, therefore, that the normal mucous membrane is capable of localizing the inflammatory process within the sacs in the majority of cases.

In the five cases in which a spreading inflammation of the mucosa had developed, the specimens showed a congested red mucosa in which there were numerous small erosions (Fig. 2). In one of these the process was very widespread and involved all the resected colon, which measured 34 cm. Abnormal sigmoidoscopic appearances were found in two cases in the rectum, and when biopsy was performed superficial erosions with lymphocytic infiltration of the lamina propria were seen. In one of these the abnormalities have persisted in the rectal stump following operation. The other two cases showed a "lumpy" appearance of the mucosal surface (Fig. 3), in addition to the superficial inflammation. This appearance was caused by a widespread lym-

Fig. 2.—Acute diverticulitis with spreading mucosal inflammation, showing numerous erosions.

Fig. 3.—"Lumpy" appearance of the mucous membrane in a case of diverticulitis.

phocytic infiltration of the submucosa and lamina propria with marked follicular hyperplasia (Fig. 4).

In these five cases with spreading lesions, the mucosal changes outside the sacs show essentially similar histological appearances. The widespread mucosal erosions do not tend to destroy entirely the crypts of Lieberkühn (Fig. 5). Regeneration can be seen going on in the same area as the inflammatory exudation. Sections through the mucosa show many crypts transected at levels where pale and flattened regenerating epithelial cells form their lining (Fig. 6). Occasionally zones of complete crypt destruction are found. In these areas repair of the type seen so frequently in ulcerative colitis can be demonstrated. Flattened epithelial cells grow from adjacent surviving crypts to cover the granulation tissue that replaces the damaged mucosa.

Studies of normal repair in the human rectum have shown that regeneration cannot occur if crypts are completely destroyed. Repair is effected by growth of flattened cells from adjacent surviving epithelium; these cells are incapable of forming new crypts (Lumb and Protheroe[3]). Similar findings in the rectum of mice have been described by O'Connor,[5] who also found that if the cells at the bases of the crypts remained intact, regeneration could occur.

The spreading mucosal changes in diverticulitis remain superficial. In some cases the inflammatory cells may infiltrate the muscularis mucosae (Fig. 5) but never spread deeply enough to involve the main muscle coats.

Quiescent Cases

Thirty-two of the surgical specimens showed evidence of chronic inflammatory changes. Mucosal abnormalities were confined to the diverticula themselves in 31 cases. In one specimen atrophic flattened mucosa was seen between the sacs and at some distance from them (Table 1).

The natural history of the disease process is an alternation of acute inflammatory episodes and periods of quiescence. The complications of abscess and fistula formation may occur at any stage. Diverticula tend to be destroyed and replaced by fibrous tissue, and in those which remain there is some flattening of the mucosa, with round-

Fig. 4.—Diffuse mucosal inflammation with follicular proliferation in a case of acute diverticulitis. Hematoxylin and eosin; × 10.

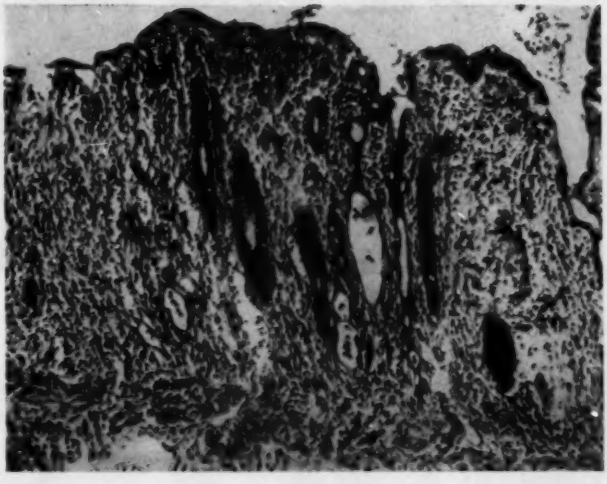

Fig. 5.—Spreading mucosal inflammation in diverticulitis, showing round-cell infiltration of the lamina propria, spreading to the muscularis mucosae. There is evidence of damaged crypts with active regeneration. Hematoxylin and eosin; × 52.

cell infiltration of the lamina propria. It may be very difficult to identify remaining diverticula when they have been destroyed completely by the inflammatory process and replaced by fibrous tissue. In unexplained inflammatory lesions in the sigmoid colon the possibility of a preexisting diverticulitis with inflammatory obliteration of the sacs should always be considered. The wall of the bowel becomes thickened, and the lumen is narrowed. Most of the inflammatory cellular proliferation is in the subserosa. The adjacent blood vessels may show obliterative endarteritis and even thrombosis. The chronic inflammatory changes are seen spreading out into the adjacent subserosa, and foreign-body giant cells associated with granulomatous proliferations may be found. Muscular hypertrophy in the bowel wall between the diverticula with proliferation of the nerve ganglion cells in Auerbach's plexus is a common finding.

The serosa is usually thickened, and there is proliferation of the vessels in this region. There is an accumulation of pericolic fat and enlargement of the appendices epiploicae, which may completely obscure the diverticula.

Of the 57 surgical specimens studied in this series, 16 showed obliterative vascular changes, 9 showed giant-cell proliferations, and in 31 there was evidence of muscular hypertrophy.

The mucosa between the diverticula showed no demonstrable abnormalities except in one case in this quiescent group, in which there was evidence of flattening with diminution of the numbers of crypts of Lieberkühn and a mild degree of lymphocytic infiltration of the lamina propria.

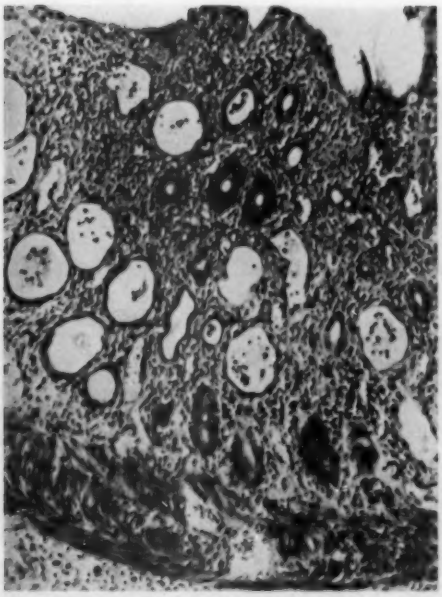

Fig. 6.—Acute spreading inflammation with regeneration of the crypts in the mucosa outside the sacs in ulcerative colitis. Hematoxylin and eosin; reduced to 88% of mag. × 96.

Fig. 7 (Case 4).—Microscopic view of specimen shown in Figure 9, showing destruction of the diverticulum, with ragged ulceration and vascular proliferation. The appearances are identical with those seen in ulcerative colitis. Hematoxylin and eosin; reduced to 76% of mag. × 10.

Cases of Diverticulitis Complicated by Ulcerative Colitis

In all four cases with complicating ulcerative colitis there had been symptoms and signs referable to the sigmoid colon which, in addition to the radiological evidence and normal sigmoidoscopic findings, had made a diagnosis of diverticulitis seem probable (Table 2). In three of them (Cases 1, 2, and 3) the subsequent course, with frequent passage of blood and mucus, sigmoidoscopic changes, and eventual spread to produce extensive involvement of the colon, made it necessary to change the diagnosis to one of ulcerative colitis. The pathological changes in these three cases show widespread mucosal destruction with infiltration of the lining epithelium by polymorphonuclear leucocytes producing so-called "crypt abscesses" (Warren and Sommers [8]), and progression to widespread, ragged ulceration (Fig. 7). Fibrosis had occurred in the wall, and diverticula were obliterated, although they had been obvious

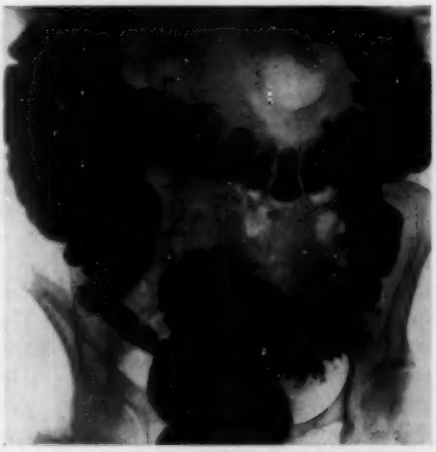

Fig. 8 (Case 1).—Radiograph showing multiple diverticula in the descending and sigmoid colon in the presence of symptoms of diverticulitis. This appearance was seen one year before the patient developed acute fulminating ulcerative colitis.

Fig. 9 (Case 4).—Diverticulitis with widespread mucosal inflammation which is indistinguishable from acute segmental ulcerative colitis and shows intense congestion and widespread ulceration.

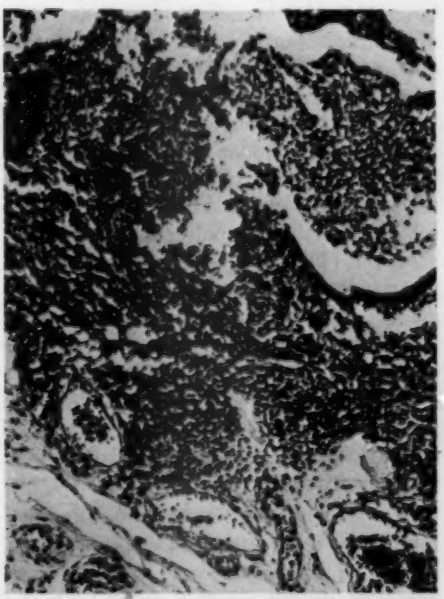

Fig. 10 (Case 4).—Acute, ragged ulceration with crypt abscess formation and gross vascular dilatation. This is an area of mucosa at some distance from the nearest diverticulum. Hematoxylin and eosin stain; reduced to 2/3 of mag. × 64.

in radiographs taken before the mucosal process began to spread (Fig. 8). The abnormalities had remained confined to the distal colon in Case 4, where the clinical picture was thought to be that of diverticulitis until the specimen was removed and examined. When the bowel was seen at operation, however, it showed generalized thickening and boggy edema of the subserosa and mesentery. When the specimen was opened after 30 cm. had been resected, the entire mucosa was seen to be reddened, and scattered over its surface were numerous small ulcers (Fig. 9). Diverticula still remain easily identifiable in this specimen, although areas of inflammatory cellular proliferation and fibrosis are seen, which probably occupy the site of preexisting diverticula. The mucosa shows a similar appearance in all four of these cases. There are widespread "crypt abscesses," large ragged ulcers, and round-cell infiltration, destroying the muscularis mucosae and spreading into the submucosa (Fig. 10). There is some evidence of epithelial regeneration at the edge of damaged areas, but only where the bases of crypts remain undamaged. Thin, flattened cells grow from these surviving crypts and cover partially the surrounding damaged areas. Small vessels are dilated but show no evidence of any primary disease or thrombosis which could have caused the mucosal damage. It is difficult to escape from the conclusion that these four cases which present a picture indistinguishable from ulcerative colitis had arisen in an area of colon where diverticulitis had previously existed. It would seem that this sequence of events is uncommon, but when it does occur, it gives rise to considerable diagnostic difficulty.

Comment

In the majority of cases of diverticulitis the inflammatory process spreads from the sac through the thin muscle strands to involve the subserosa early in the process. Mucosal involvement is usually localized within the sacs, but when spread to the

mucous membrane between the diverticula occurs, epithelial regeneration is common because the crypts of Lieberkühn are only partially destroyed.

This tendency to mucosal regeneration is markedly different from the changes which occur in ulcerative colitis and which were seen in the four cases of this series in which this process complicated diverticulitis. In this disease the process is severer and complete crypts are destroyed with the production of definite ulcers, which tend to spread. Quiescent periods are common when the inflammatory process subsides, but during these periods there is always microscopic evidence of a damaged, inadequately healed mucosa even when its appearances may seem to be normal as viewed by the naked eye (Lumb and Protheroe[3]).

In recent years many workers have noted the association of carcinoma of the colon and rectum and ulcerative colitis. Bargen, Sauer, Sloan, and Gage,[7] basing their observations on a series of 1500 cases of ulcerative colitis seen at the Mayo Clinic, stated that carcinoma is 20 to 30 times commoner as a cause of death in this disease than in the population as a whole. It seems probable that malignant change occurs more frequently in those who have had colitis for many years (Counsell and Dukes[8]; MacDougall[9]).

Although the clinical differentiation between carcinoma of the colon and diverticulitis may present great difficulty, there is no evidence for anything more than a coincidental relationship between the diseases (Stewart[10]). In the 168 cases of diverticulitis available for study in our hospital, carcinoma occurred in 3. In the 57 surgical specimens examined there was a coincidental finding of adenomatous polyps in 6 cases, one of which showed superficial anaplastic changes. The tumors did not arise in diverticula but had origin from mucosal surfaces between the sacs, which showed no evidence of any underlying abnormality.

It seems possible that the difference in the mucosal damage between ulcerative colitis and diverticulitis may have some bearing on the relationship of these conditions to carcinoma. In ulcerative colitis large areas of mucosa are involved, and destruction and repair continue over a long period. In diverticulitis the area of mucosa involved is small and damage only rarely proceeds beyond a point where regeneration is possible.

Most authors have stated that the differential diagnosis of diverticulitis of the distal colon and ulcerative colitis presents very little of a problem. It is true that when typical examples of the two diseases are seen, marked differences, such as the age of onset, the radiological appearances, and the clinical course, make the diagnosis obvious. When diverticulitis is associated with acute spreading mucosal lesions, or when ulcerative colitis affects an isolated zone of the sigmoid colon, diagnostic problems may arise. This is particularly true if the patient is over 45 years of age. Cases 1 to 4 (Table 2) in this series illustrate this point. A case in which the clinical diagnosis was diverticulitis, but which showed the appearances of ulcerative colitis of a segmental type when the specimen was examined, is Cabot Case 38402.[11] The appearances seem to have been very similar to those found in our Case 4, in which a clinical diagnosis of diverticulitis was changed to one of segmental colitis when the sigmoid colon was seen at operation (Fig. 9). Morton[12] has reported diverticulitis with ulceration of the rectal mucosa. Inflammatory changes with erosions were noted in the rectum on two occasions in this series. It is interesting to speculate whether diverticulitis with widespread mucosal involvement may progress to produce a condition indistinguishable from ulcerative colitis.

No satisfactory "cause" of ulcerative colitis has been suggested. There are a number of inflammatory or traumatic conditions in the colon which usually subside, leaving no permanent structural abnormality. Occasionally, they may act as a "trigger" mechanism which starts a progressive ulcer-

ative lesion. A large number of factors have been described as predisposing to ulcerative colitis. Thus, Felsen [13] was of the opinion that bacillary dysentery may precede ulcerative colitis; Grace, Wolf, and Wolff [14] have invoked parasympathetic hyperactivity originating from psychogenic stimuli, and Andresen * has stressed the possibility of some allergic type of inflammatory origin.

In the majority of the cases of diverticulitis which we have examined the mucosal changes have remained localized within the diverticula. In those acute cases in which some inflammatory spread had occurred to involve the mucosa between the sacs, damage to the crypts of Lieberkühn was not so severe as is seen in ulcerative colitis and there was evidence of very considerable regeneration. In only 1 of the 32 quiescent cases were there any demonstrable mucosal abnormalities. Four cases were found, however, where the widespread destructive changes of ulcerative colitis appeared to have supervened in the course of diverticulitis. It seems possible that diverticulitis may have acted as the "trigger" mechanism in this small group where there had been an inability to localize the infection.

Summary

The appearances of the mucosa in diverticulitis have been investigated in order to study the degree of localization of the inflammatory process and to compare them with the changes in ulcerative colitis.

Of 57 surgical specimens examined, 47 showed no evidence of any abnormality in the mucous membrane between the diverticula, 5 specimens showed spreading mucosal changes with evidence of regeneration, 1 showed flattened atrophic mucosa between the diverticula, and 4 cases showed changes indistinguishable from ulcerative colitis.

The mucosal changes in diverticulitis are discussed in relation to carcinoma and ulcerative colitis.

Members of the medical and surgical staffs of the Westminster and Gordon Hospitals collaborated in this study; Dr. P. Hansell, Director of the Department of Medical Photography of Westminster Hospital, made some of the photographs, and Mr. J. Stokes gave technical assistance with the histology and photomicrography.

One of us, R. H. B. P., was assisted in this investigation by a grant from the Westminster Hospital Board of Governors Discretionary Fund.

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Utilization of S^{35}-Labeled Sulfate in Scorbutic Guinea Pigs

Uptake in Healing Wounds, Megakaryocytes, and Blood Platelets

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The disturbance of wound healing which is characteristic of scurvy has been ascribed to a disorder in the formation of granulation tissue; it has been repeatedly noted that, although fibroblasts are capable of proliferation, collagen fibers are not formed (Wolbach and Howe, 1926; Wolbach, 1933). Histochemical studies have pointed toward a disturbance in the production of mucopolysaccharides (Penney and Balfour, 1949; Gersh and Catchpole, 1949), and hydroxyproline (Dunphy and Udupa, 1955) in the intercellular ground substance, but the precise site and mechanism of the disorder are as yet unknown. The defective production of new connective tissue appears to be associated also with faulty maintenance of preformed mesenchymal structures, as indicated by capillary fragility (Ralli and Sherry, 1941), which is accompanied by a tendency toward thrombocytopenia (Presnell, 1934).

Because S^{35}-labeled sulfate has been found to be selectively incorporated into the mucopolysaccharide and collagen of normal fibrous connective tissue (Dziewiatkowski and associates, 1949; Odeblad and Boström, 1952; Davies and Young, 1954) and into megakaryocytes and platelets (Odell and associates, 1955), the present study was undertaken to investigate the uptake of S^{35}-labeled sulfate in the healing wounds, megakaryocytes, and platelets of scorbutic guinea pigs.

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Materials and Methods

An experimental group of 10 young adult guinea pigs, weighing 350-500 gm., was fed a scorbutigenic diet of ground Purina Laboratory Chow without added ascorbic acid or green vegetables; the 10 control animals were fed the Purina Chow and fresh lettuce. Drinking water was always present in the cages. Twelve days after the scorbutigenic regimen was started, a small amount of extensor muscle was excised from a thigh of each experimental animal, and the wound was sutured. Four days later, half the animals from each group were injected intraperitoneally with 170 μc per kilogram of body weight of S^{35}-labeled sodium sulfate in approximately 0.3 ml. of isotonic saline solution; nine days after operation the remainder of the animals were similarly injected. Twenty-four hours after injection, the guinea pigs were given 0.05 mg. of heparin (in 0.5 ml. of saline) intravenously, and bled to death under pentobarbital anesthesia 15 minutes later. Specimens of healing wound tissue and femoral bone marrow were fixed in 95% ethanol, embedded in paraffin, sectioned at 5μ, and processed for autoradiography by the method of Gude and associates (1955). Bone marrow smears were also prepared by the technique of Gude and Odell (1955). Sections were stained with hematoxylin and eosin, 1:100,000 aqueous toluidine blue, by the periodic acid-Schiff procedure, and by the silver nitrate impregnation for reticulum. Platelets were counted just before killing by the method of Brecher and Cronkite (1950). At exsanguination, blood from the abdominal aorta was collected in a silicone-coated syringe containing 0.5-1.0 ml. of edathamil (Sequestrene, 1% in 0.7% saline) and transferred immediately to a chilled centrifuge tube. The platelets were then separated by differential centrifugation, washed, and suspended in isotonic saline solution according to a modification of the technique of Dillard and associates (1951). Samples of platelet suspensions and blood plasma

were placed on planchets and allowed to dry, and their radioactivity assayed in a proportional gas-flow counter.

Results

The wounds of the nonscorbutic guinea pigs exhibited active fibroblastic proliferation and were moderately well organized at the periphery by the fifth day (Table 1). Abundant intercellular ground substance that stained metachromatically with dilute toluidine blue was present at this time. Maturing fibroblasts were surrounded by numerous reticulin and collagen fibers, as shown by silver impregnation (Fig. 1). Autoradiograms (30-day exposure) revealed incorporation of radioactive sulfur in the newly formed connective tissue, which appeared to be concentrated in regions of active intercellular fiber formation (Fig. 2). Although the site of the radioactivity could not be located with certainty, the density of photographic grains appeared to be relatively greater over the areas surrounding and between the fibroblasts than directly above the cells themselves, suggesting that the S^{35} was predominantly extracellular in location. Only relatively small amounts of radioactivity were observed in primitive fibroblasts in areas devoid of

TABLE 1.—*Incorporation of Sulfur 35-Labeled Sulfate in Wounds of Normal and Scorbutic Guinea Pigs*

Treatment Group	Uptake of S35-Labeled Sulfate*			Fibroblastic Proliferation	Relative Amount of Metachromatic Ground Substance	Formation of Reticulin Fibers	Collagen Formation
	Fibroblasts	Intercellular Space					
		Afibrillar	Fibrillar				
Scorbutic	±	±	±	++	±	0	0
Nonscorbutic	+	+	+++	+++	++	+++	++

* As judged in autoradiograms. Assays of wound tissue also suggested a greater sulfate uptake in nonscorbutic than in scorbutic guinea pigs (radioactivity of sulfate in wound was counted as $BaS_{35}O_4$ after fusion of the tissue with Na_2CO_3, neutralization with HCl, and precipitation with $BaCl_2$).

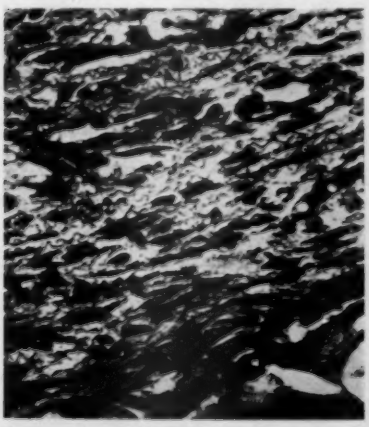

Fig. 1.—Granulation tissue in a five-day-old wound of a nonscorbutic guinea pig. Maturing fibroblasts are surrounded by numerous reticulin fibers. Silver nitrate impregnation; × 475.

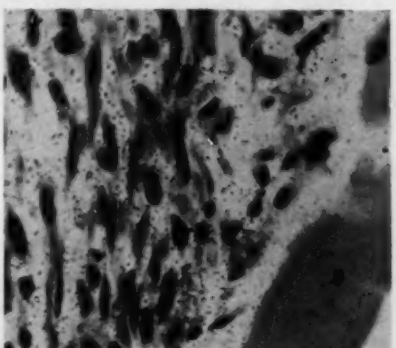

Fig. 2.—Stripping-film autoradiogram of a five-day-old wound of a nonscorbutic guinea pig (exposed for 30 days). Photographic grains in the overlying emulsion are concentrated at the site of collagen formation. Giemsa stain; × 475.

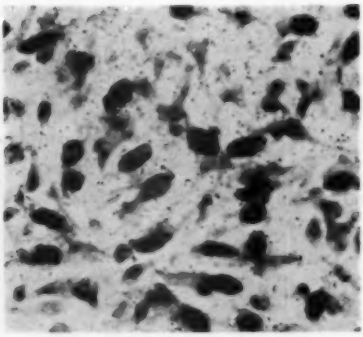

Fig. 3.—Stripping-film autoradiogram of a five-day-old wound of a nonscorbutic guinea pig. Immature fibroblasts in a region of young granulation tissue exhibit relatively little radioactivity. Giemsa stain; × 475.

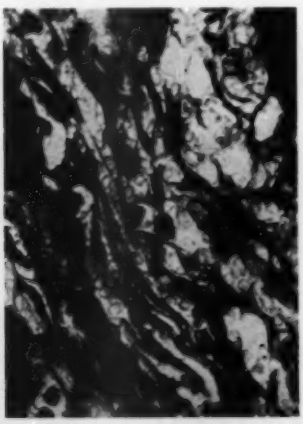

Fig. 4.—Granulation tissue in a five-day-old wound of a scorbutic guinea pig. Fibroblasts are relatively immature, and intercellular fibers are lacking. Silver nitrate impregnation; × 475.

ground substance or reticulin fibers (Fig. 3). Immature fibroblasts containing abundant intracytoplasmic PAS-positive material were occasionally observed, such as those shown in Figure 5; in PAS-stained autoradiograms these cells did not appear radioactive.

Fibroblasts from the wounds of the scorbutic guinea pigs were relatively immature and the wounds lacked organization 5, (Fig. 4), or even 10, days after operation. Metachromatic intercellular ground substance was virtually absent, and no newly formed reticulin or collagen fibers

Fig. 5.—Fibroblasts in a 10-day-old wound of a scorbutic guinea pig, which contain abundant intracytoplasmic PAS-positive material. Periodic acid-Schiff (PAS); × 925.

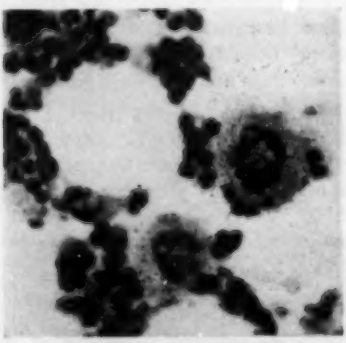

Fig. 6.—Stripping-film autoradiogram of a section of femoral bone marrow from a normal guinea pig. Megakaryocytes exhibit a high selective concentration of radioactive sulfur, as indicated by the density of grains in the overlying emulsion. Giemsa stain; × 475.

were observed. Autoradiograms indicated only relatively small amounts of activity in the granulation tissue in the location of the more mature fibroblasts. Many primitive fibroblasts that contained abundant PAS-positive material in the cytoplasm were noted (Fig. 5); these cells exhibited little or no evidence of radioactivity in autoradiograms.

Blood of the scorbutic animals contained fewer platelets than that of the controls, and the specific activity of these platelets was slightly lower (Table 2). Autoradiograms

TABLE 2.—*Uptake of Sulfur 35—Labeled Sulfate In Platelets*

Treatment Group	Av. No. of Platelets per Cu. Mm. of Blood×10³	Specific Activity	
		Cts/Min/Av/ Platelet×10⁷	Cts/Min/100µl of Plasma
Scorbutic	462 (365–565)*	4.7 (3.7–5.8)	2072 (1778–2709)
Normal	773 (680–865)	6.3 (5.7–7.0)	2858 (2456–3468)

* Numbers in parentheses indicate the range of values per group.

of bone marrow (30- to 80-day exposure) indicated activity in megakaryocytes (Fig. 6) but none in other hematopoietic elements.

Comment

The incorporation of radiosulfate into the newly formed connective tissue of healing wounds may conceivably be correlated with the formation of sulfonated mucopolysaccharides in the intercellular ground substance and collagen (Meyer and Rapport, 1951). A high uptake of S^{35}-labeled sulfate has been observed in several tissues rich in sulfomucopolysaccharides (Dziewiatkowski, Benesch, and Benesch, 1949; Layton, 1951; Odeblad and Boström, 1952; Davies and Young, 1954; Reddi and Nörstrom, 1954); furthermore, when given to the rat in the form of sulfate, sulfur does not enter appreciably into compounds such as methionine and cystine (Dziewiatkowski, 1954; Tarver and Schmidt, 1939).

The utilization of sulfate in the formation of connective tissue has been discussed by Glucksmann and associates, who studied the uptake of radiosulfate in healing wounds and mature connective tissue in adult mice. These investigators observed incorporation of sulfate into fibroblasts preceding and during fiber formation; however, with the production of intercellular fibers, concentration of sulfate appeared to shift from within the fibroblast to the extracellular space. Although the source of the intercellular mucopolysaccharide is not known, these observations support the concept that it is of fibroblastic origin, as postulated by Gersh and Catchpole (1949), who observed glycoprotein in proliferating fibroblasts; this has also been noted by others (Coon and Upton, 1952). It appears significant, however, that in the present experiments such material was not found to contain S^{35}, and the relative amount of radioactivity in granulation tissue, as judged in autoradiograms, was maximal during fiber formation. These results are in keeping with the observation that the ratio of hyaluronic acid to chondroitin sulfate in human skin decreases with the formation of collagen fibers (Watson and Pearce, 1950).

The lack of uptake of radiosulfate in the wounds of the scorbutic animals is consistent with the diminished incorporation of sulfate observed in most mesenchymal tissues of the guinea pig during scurvy (Friberg and Ringertz, 1954). Kodicek and Loewi (1955) also noted a marked reduction in the uptake of S^{35}-labeled sulfate by the granulation tissue of regenerating tendon in the scorbutic guinea pig, despite the presence of normal amounts of polysaccharide in the wound, and inferred that scurvy interfered with the process of sulfonation. Sulfonation was also inhibited in vitro by disruption of the cells or by metabolic poisons, such as 2, 4-dinitrophenol, azide, cyanide, and certain sulfhydryl-binding substances, suggesting that the uptake of sulfate by granulation tissue depended on an enzymatic process that possibly involved oxidative phosphorylation.

The lack of sulfate incorporation in immature normal and scorbutic fibroblasts, including those containing abundant PAS-positive glycoprotein, suggests that sulfonation must occur at a relatively late stage in the maturation of the fibroblast, presumably immediately preceding and during fiber formation. If, therefore, sulfonation is considered to take place in the second, or

"collagen," phase of wound healing, rather than in the initial "substrate," or "productive," phase, during which mucopolysaccharides accumulate (Dunphy and Udupa, 1955), the results of the present investigation are consistent with the prolongation of the "substrate" phase and absence of the "collagen" phase, which have been observed in scurvy (Dunphy and Udupa, 1955).

The incorporation of radiosulfate by platelets and megakaryocytes has been noted previously in rats, and the labeling sequence of these cells suggests that the tagged platelets were derived from labeled megakaryocytes (Odell and associates, 1955). The chemical nature of the S^{35} in these cells is yet to be determined; however, cytochemical investigations point toward the presence of a mucopolysaccharide in platelets (Gude, Upton, and Odell, 1955) that may contain the sulfate. The subnormal concentration of radiosulfate observed in the scorbutic platelets is difficult to evaluate in the light of the relatively low level of radioactivity noted concomitantly in the plasma; if corrected for the low plasma level, however, the sulfate uptake of the scorbutic platelets would not appear to be significantly depressed. The reduced plasma concentration of S^{35} in the scorbutic animals is unexplained.

Summary

1. Relatively little uptake of S^{35}-labeled sulfate was detected autoradiographically in 5- or 10-day-old wounds of scorbutic guinea pigs, whereas relatively large amounts were concentrated in healing wounds of nonscorbutic animals.

2. The location of the sulfate in the granulation tissue appeared to be predominantly extracellular at the site of fiber formation; however, small amounts of sulfate were observed in normal, and scorbutic, fibroblasts preceding the production of reticulin fibers.

3. Immature fibroblasts, including those containing abundant PAS-positive intracytoplasmic material, gave no evidence of sulfate incorporation.

4. Radiosulfate was concentrated in megakaryocytes and platelets, its uptake by these elements appearing to be slightly, if at all, depressed in scorbutic animals.

Mrs. F. G. Tausche, Mr. W. D. Gude, Mrs. F. F. Wolff, and Mr. T. Mack gave technical assistance.

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Adrenal Cortex and Hepatic Cirrhosis

I. Role of Adrenal Cortex in Evolution of Carbon-Tetrachloride-Induced Cirrhosis

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Carbon tetrachloride has been shown by Cameron and Karunaratne [6] to be capable of inducing cirrhosis in albino rats if injected at sufficiently close intervals for some time. The authors made an important observation, that this cirrhosis is reversible up to a certain stage and only later does it become permanent and progressive. This aspect has aroused a great deal of interest and has since then been studied from the point of view of neoangiogenesis,[15] alterations in ground substance,[8] and histochemical changes.[20] Balasubrahmanyan [3] found no significant qualitative changes in the ground substance at various stages of carbon-tetrachloride-induced cirrhosis. Cameron [7] has cautioned against the supposed infallibility of the injection techniques. The striking divergence of opinion on the architecture and vascular pattern of liver arrived at by Elias [10] and Rappaport and associates,[16] using the injection techniques, also bespeaks the limitations of this method.

Recently Selye [19] has stated that chronic stress can result in the development of cirrhosis. Hill [12] postulated the role of adrenal cortex in the pathogenesis of "serous hepatosis," though he produced no evidence to support this view. Aterman [2] obtained good results in clinical, as well as experimental, cirrhosis with cortisone therapy. Brown [5] and Bluemle [4] and their associates noted reduction in the fibrosis and fatty infiltration of the liver after corticotropin. Cortisone in pharmacological doses, however, may be effective in ameliorating signs and symptoms of various lesions, even though the adrenals may not be unequivocally involved in their pathogenesis. Contrarily, Diengott and Ungar [9] have observed that cortisone therapy may actually aggravate the cirrhosis produced by chronic carbon tetrachloride injury.

The present study was undertaken to gather evidence, direct as well as indirect, (1) as to whether the evolution, and especially the stage of irreversibility, of carbon-tetrachloride-induced cirrhosis is conditioned by the abnormality of adrenal function, and (2) whether cortisone has an ameliorating or accentuating effect on the cirrhotic process at its various stages.

Materials and Methods

A total of 300 young albino rats of both sexes, weighing 80-200 gm., were used. Of these, 250 animals were injected twice a week each with 0.1 cc. of carbon tetrachloride per 100 gm. of body weight in an equal volume of light liquid petrolatum N. F. subcutaneously. A batch of seven to nine rats were killed at weekly intervals, from the third week onward, and the extent of cirrhosis was studied. The time the animals were killed was so arranged that it was 60-64 hours after the last injection of carbon tetrachloride. From the sixth week onward a batch of eight rats was isolated every week to which no further injections were given. From these animals for follow-up studies, batches of four rats each were killed after two and three weeks respectively, to study the reversibility or otherwise of the cirrhotic process. Cirrhosis, which was seen to be receding appre-

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ciably in a maximum of three weeks, was considered reversible.

The animals were killed by stunning. The liver was resected and both the adrenals taken out. Slices of liver were fixed in 10% formalin. They were embedded in paraffin and the sections stained with hematoxylin and eosin, Masson's trichrome stain for connective tissue, and the reticulum stain by the silver impregnation technique of Gomori. Representative pieces from the various lobes of the liver were minced and their collagen content was estimated by the technique of Lowry and associates.[14]

Adrenal function was assessed by a study of the ascorbic acid content, sudanophilia, histological features of the adrenal cortex in the hematoxylin and eosin stain, and a histochemical study for 17-ketosteroids. Of the two adrenals, one was fixed for 48 hours in neutral formol-saline and washed for 24 hours in running water, and frozen sections were cut. The sections were stained with hematoxylin and eosin, for sudanophilia by the technique of Chiffelle and Putt,* and for 17-ketosteroids by the technique of Albert and Leblond.[1] The ascorbic acid content was estimated by the technique of Roe and Kuether* after homogenizing the adrenal in a solution of 5% *m*-phosphoric acid in 10% acetic acid.

With each weekly "forward" experiment,† two animals of the same batch were exposed to a non-specific stress of low temperature, by putting them on ice for three and a half hours, at the end of which the animals were killed and their adrenals studied for the ascorbic acid content. Another two rats each were given subcutaneous injections of histamine hydrochloride (1 mg.) and corticotropin (1 mg.) to subject them to indirect, as well as direct, cortical stimulation. These rats were also killed after three and a half hours, and the ascorbic acid content of the adrenals was estimated. These types of stresses were designed to investigate whether the pituitary and adrenal cortices, singly or collectively, were participating properly in the adaptation mechanism.

Since the rats of the "forward" experiments were killed, roughly, about 60 hours after the last injection of carbon tetrachloride, a control experiment to obtain comparable ascorbic acid values in the normal rat after giving a similar dose of the chemical was run by killing 30 rats in five batches at varying intervals of 3½ to 60 hours after the injection. At 60 hours, four rats were injected again with 0.1 cc/100 gm. of body weight of carbon tetrachloride, and another four each were exposed to the stress of cold, histamine, and corticotropin for 3½ hours and the ascorbic acid content of the adrenals estimated. The effect of similar types of stress on four normal, uninjected animals was also studied to serve as the control.

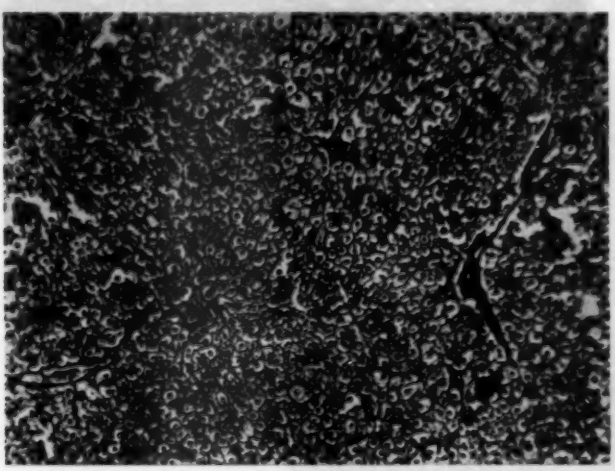

Fig. 1. — Liver showing Grade 1+ fibrosis. Reticulum stain; × 100.

* Cited by Hawk and others.[17]

† The experiment in which a batch of animals was killed weekly during the course of the injections, beginning three weeks after the start of the injections.

Observations

Histological Studies of the Liver

In this experiment, in which the reversibility of cirrhosis was the moot point under discussion, it was considered essential to classify the severity of the cirrhotic process under five arbitrary grades for purposes of assessing the progression or

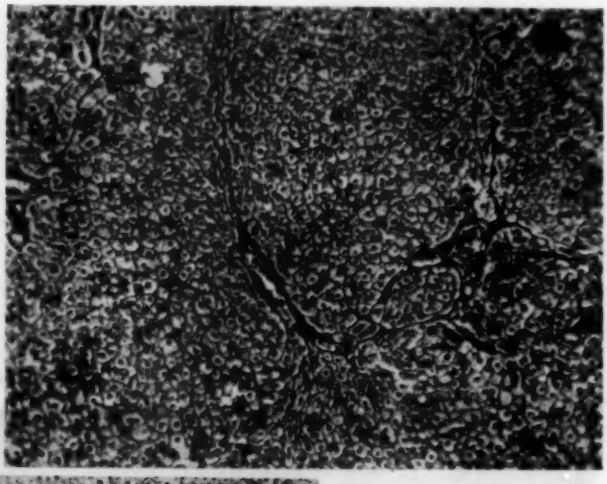

Fig. 2. — Liver showing Grade 2+ fibrosis. Reticulum stain; × 100.

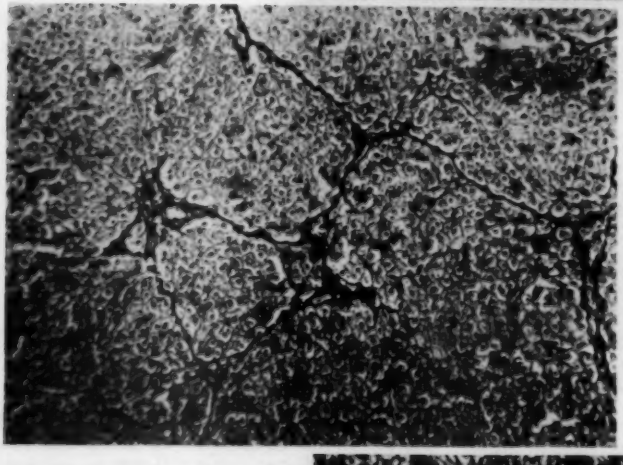

Fig. 3. — Liver showing Grade 3+ fibrosis. Reticulum stain; × 100.

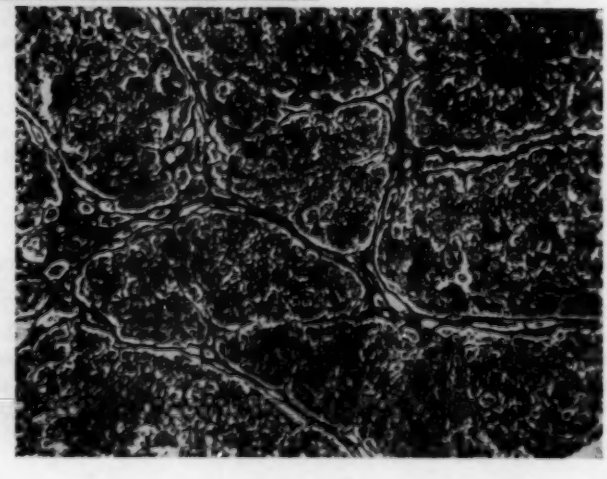

Fig. 4. — Liver showing Grade 4+ fibrosis. Reticulum stain; × 100.

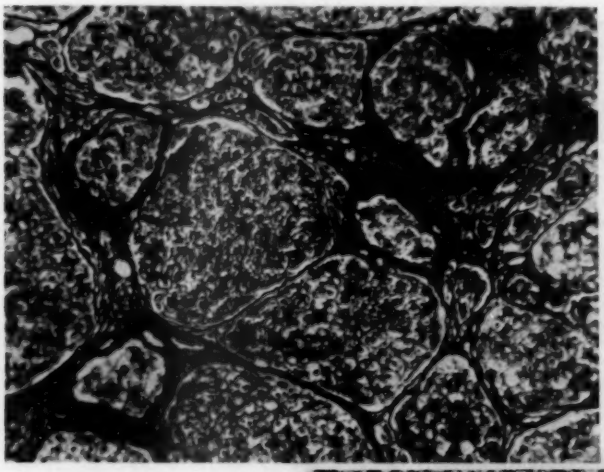

Fig. 5.—Liver showing 5+ fibrosis. Reticulum stain; × 100.

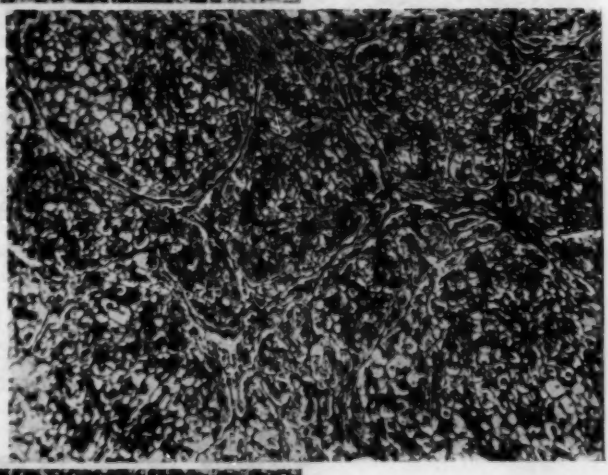

Fig. 6.—Sixth week. Liver showing severe degree of cirrhosis. Reticulum stain; × 100.

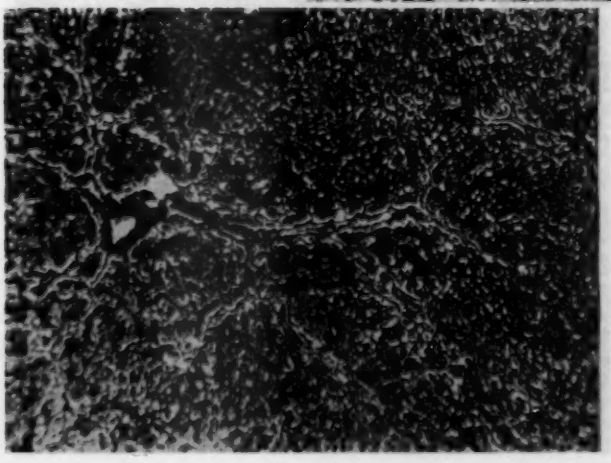

Fig. 7.—Sixth week: first follow-up (two weeks). Liver showing considerable regression of fibrosis. Reticulum stain; × 100.

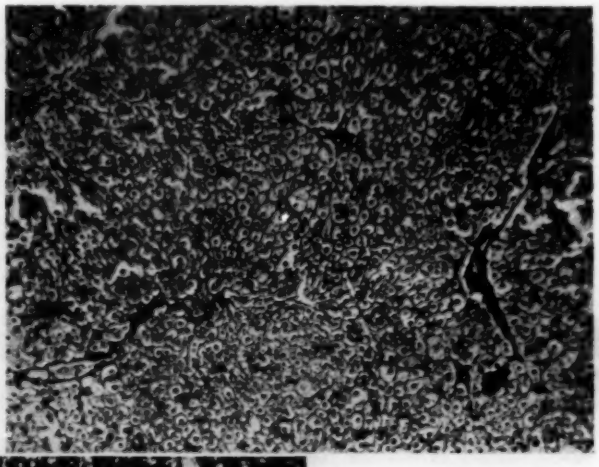

Fig. 8.—Sixth week: second follow-up (three weeks). Liver showing almost complete reversal of cirrhosis. Reticulum stain; × 100.

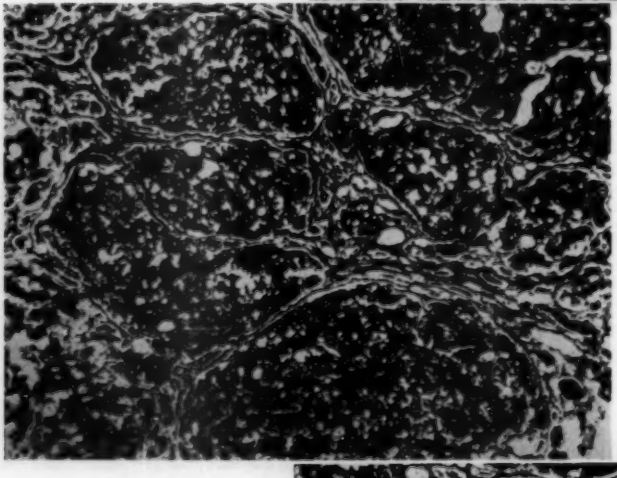

Fig. 9.—Tenth week: liver showing severe degree of cirrhosis. Reticulum stain; × 100.

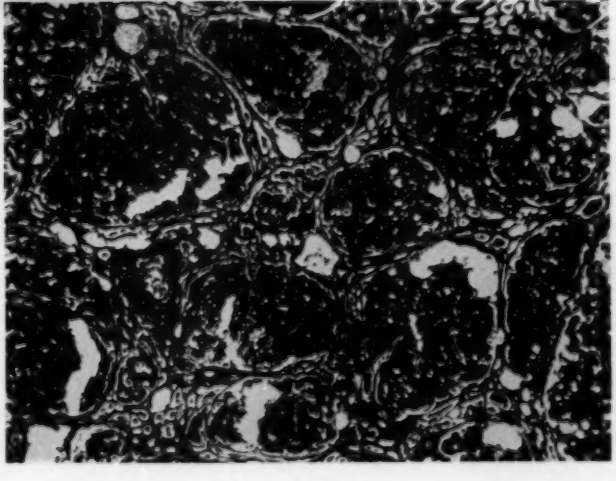

Fig. 10.—Tenth week: first follow-up (two weeks). Liver showing no regression of fibrosis. Reticulum stain; × 100.

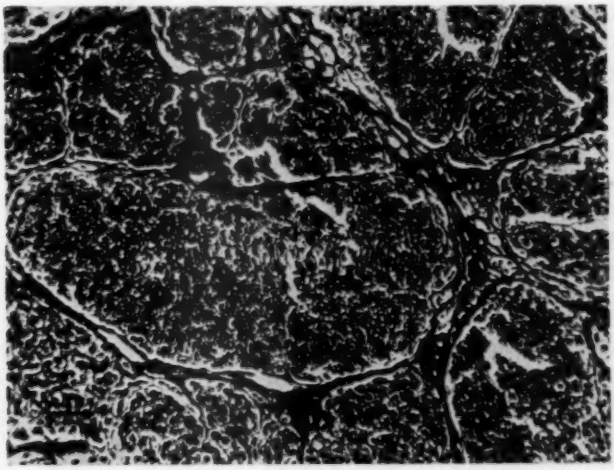

Fig. 11. — Tenth week: second follow-up (three weeks). Liver showing irreversible cirrhosis. Reticulum stain; × 100.

TABLE 1.-*Average Grades of Fibrosis and Collagen Content of the Livers in the "Forward" and Follow-Up Groups**

Week	"Forward"		First Follow-Up		Second Follow-Up	
	Fibrosis (Average Pluses)	Collagen Mg/Gm.	Fibrosis (Average Pluses)	Collagen Mg/Gm.	Fibrosis (Average Pluses)	Collagen Mg/Gm.
3	0.5 (0–1)	6.44 (4.87–7.65)				
4	1.5 (1–2)	6.82 (6.42–7.23)				
5	1.75 (1–2)	7.05 (5.12–9.4)				
6	2 (1–4)	8.61 (7.56–9.25)	1 (1–1)	6.61 (5.98–7.25)	0.5 (0–1)	6.44 (5.76–7.26)
7	2 (2–2)	8.55 (7.52–9.52)	0.5 (0–1)	7.14 (6.98–7.31)	0.5 (0–1)	7.09 (6.96–7.56)
8	3 (1–4)	12.69 J (8.9–14.89)	2.3 (2–3)	6.52 (5.12–7.31)	0.5 (0–1)	6.06 (5.21–6.95)
9	4.1 (2–5)	17.91 (12.13–27.62)	2.5 (2–3)	8.3 (4.19–12.71)	2.2 (1–3)	7.52 (5.53–9.70)
10	4.3 (3–5)	21.49 (13.6–26.75)	4.5 (4–5)	20.97 (15.97–26.05)	4 (4–4)	14.51 (10.86–20.37)
11	4.64 (4–5)	24.71 (23.3–25.72)	4.2 (3–5)	21.15 (11.38–27.41)	4 (3–5)	19.73 (10.96–30.3)
12	5 (5–5)	26.48 (23.24–30.34)	5 (5–5)	25.6 (21.37–28.39)	4.5 (4–5)	25.5 (24.76–2 .25)
13	4.75 (4–5)	28.61 (27.69–29.21)	5 (5–5)	27.53 (26.3–28.76)	5 (5–5)	27.92 (27.52–28.32)
14	4.85 (4–5)	31.31 (22.72–36.32)	4.5 (4–5)	30.74 (26.59–34.89)	5 (5–5)	32.83 (32.18–32.8)

* Figures in parentheses indicate the range.

regression of cirrhosis. These grades are represented in the photomicrographs (Figs. 1 to 5), which are self-explanatory. The reticulum stain was felt to be the most reliable, as it showed changes from the earliest condensation of reticulum down to the formation of thick collagenous bands. Essentially the thickness and extent of the

trabeculae and the degree of pseudolobulation were taken into account.

Table 1 represents the degree of fibrosis at varying periods of the experiment, correlated with the collagen content of the liver. From the point of view of cirrhosis, the earliest change did not affect the connective tissue of the liver to any recognizable extent. It was only after three to four weeks that reticulum condensation appeared. At this stage, the Masson trichrome stain revealed equivocal increase in fibrous tissue. The slender connective tissue septa were, however, cellular. At the fifth week the earliest evidence of neoangiogenesis was seen. At this period the reticulum proliferation was much more marked than the connective tissue proliferation in Masson's stain. In the follow-up studies, the regression of fibroplasia and lobular reconstitution were complete at the end of three weeks. The same qualitative changes were more or less present in the seventh-eighth-week series (Figs. 6 to 8).

Quantitatively, the changes were more marked in the later periods, some livers showing greater distortion, and even pseudolobulation, in the eighth week. At the ninth week the cirrhotic process was distinctly severer, most of the livers showing a 4+ to 5+ grade of cirrhosis. However, there was a notable regression of fibrosis, as at the end of three weeks the grades ranged from 1+ to 3+ only. The fibroplasia was not wholly cellular, but partly fibrous as well. At the 10th week the changes were all the more marked. They also tended to persist in the follow-up studies, and even became slightly more accentuated in the second- and third-week follow-ups (Figs. 9 to 11). In the subsequent batches, e.g., in the 11th- to 14th-week periods, cirrhosis was further aggravated. Lobular distortion, fibroplasia, neoangiogenesis, etc., not only stayed in the follow-ups but also distinctly progressed. The fibrous tissue septa were, however, less cellular.

Collagen Content of the Livers

Microscopic examination alone may fail to give a correct assessment of the extent or disappearance of cirrhosis, as study of a histological section gives only a one-plane view of the morphologic change. Warren and Wahi,[21] in their study of the relationship of the fibrous tissue content to the histological appearance of pathologic livers, found that no linear correlation existed between the histologically and chemically demonstrable fibrous tissue of the cirrhotic livers when fibrosis was far advanced. Hence, in an investigation in which it was important to know of the slight increase or decrease of fibrosis, it was also considered essential to include a study of the collagen content estimated chemically.

Table 1 gives the relationship of the average grades of fibrosis to the collagen content of the livers at the various periods in the "forward" and in the follow-up studies. Our normal collagen content for the control animals was 5.13 mg/gm. of liver, ranging from 2.81 to 6.66 mg/gm. of liver. It may be seen that there was almost no increase of collagen in the third to the fourth week, From the fifth week onward there was some tendency to rise, but the collagen content came down to well within normal limits up to the eighth week in the follow-up studies. This was also correlated with the histological disappearance of fibrosis. From the ninth week the fibrosis tended to persist and showed an almost linear increase in the second follow-ups in the subsequent weeks. It is of note that histologically at all these periods the fibrosis was 4+ to 5+, thus indicating the fallibility of histological examination alone. In all periods except the 14th week, there was a minor reduction of collagen in the follow-ups as compared with the "forward" groups. Thus, by and large, the entire process is divisible into four more or less distinct phases: (1) no cirrhosis (up to 5th week); (2) reversible cirrhosis (6th to 8th week); (3) irreversible cirrhosis (9th to 11th week); (4) irreversible and progressive cirrhosis (12th week and later).

Ascorbic Acid Studies of the Adrenal Cortex

Ascorbic acid values of normal young albino rats ranged from 1.23 to 1.36 mg/gm. of adrenal tissue, with a mean of 1.3125 mg/gm. and a standard deviation of 0.03441, a standard error of 0.0086, and a coefficient of variation of 2.62% (Table 2).

Cold stress continued for three and a half hours resulted in an average of 77% depletion of ascorbic acid of the adrenals, with a range of 72% to 79%. Injection of 0.1 cc. of carbon tetrachloride resulted only in an average of 55.2% depletion after three and a half hours. This shows that carbon tetrachloride is a weaker stress for eliciting the general adaptation syndrome. Histamine resulted in an average of 52% depletion (49% to 54%), and corticotropin in 59% depletion (54% to 62%) in the same time (Table 2).

Observations at different periods with regard to ascorbic acid values after a single injection of 0.1 cc. of carbon tetrachloride revealed that at 6½ hours the values had started to rise and 60 hours after injection their average value was 1.85 mg/gm., which was 141.2% of the average normal, a value which is significantly higher. A cold stress given for three and a half hours at this stage resulted in lowering of the ascorbic acid levels to 0.49 mg., i.e., in 73.95% depletion, whereas 0.1 cc. of carbon tetrachloride injected resulted only in a 24% lowering in a similar period. Chart 1 depicts the average behavior described above with regard to the ascorbic acid levels.

Table 2 and Chart 2 give the ascorbic acid values of the adrenal at different stages of the cirrhotic process and changes in its

TABLE 2—*Ascorbic Acid Content of the Adrenal Cortex at Varying Periods and Its Depletability by Various Types of Stress*

No. of Week	Adrenal Ascorbic Acid, Mg/Gm.	Adrenal Ascorbic Acid, % of Normal	Depletability of Ascorbic Acid, % of Normal		
			Cold	Histamine	Corticotropin
Normal	1.3125±0.0825	100	77	52	59
1	1.57±0.1	120	77.2	53.1	60
2	1.50±0.16	114.5	62	51	59
3	1.41±0.09	108.2	73.3	54	57
4	1.54±0.11	117.6	68	52.5	58
5	1.45±0.12	110.7	64	50	57
6	1.00±0.11	76.3	62	51	54
7	0.93±0.14	71.45	66	49	55
8	0.96±0.09	73.28	60	49	57
9	1.04+0.1	79.4	45	43	54
10	1.07±0.15	82.06	42	40	43
11	1.18±0.15	90.67	42	22	28
12	1.49±0.06	113.7	34.4	28	32
13	1.48±0.1	113.3	40	27	38
14	1.5 ±0.07	114	40	26	42

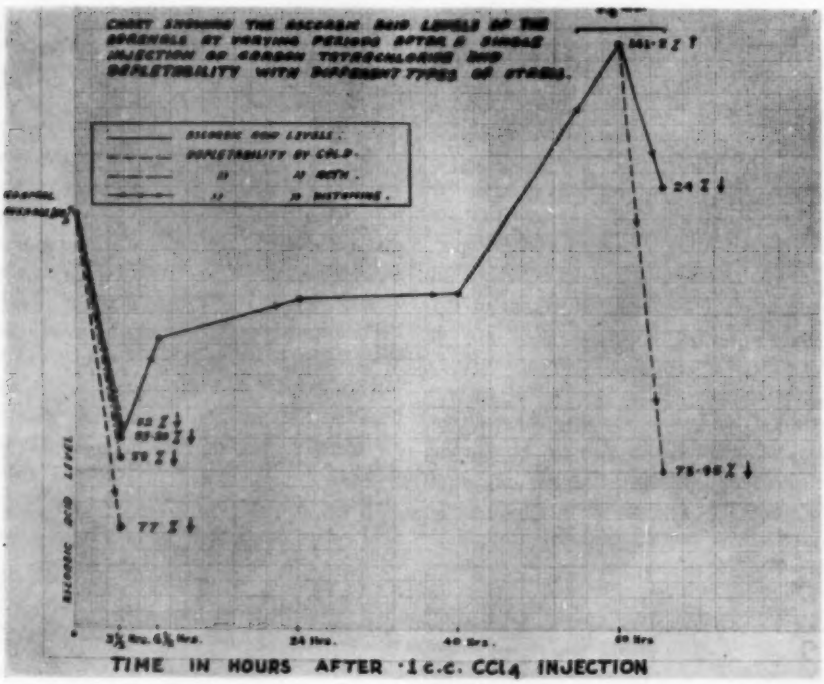

Chart 1

depletability following the three types of stress at these periods.

Generalizing from the above data, the observations in the four phases can be described as follows:

Phase I extends roughly up to the fifth week after starting the injections. In this phase the ascorbic acid at 60 hours after the last injection showed average values which were significantly higher as compared with the average normal, i.e., more than 3 S.D. higher than the normal mean. These values were also capable of being induced to practically normal diminution by exposure to all the three types of stresses for three and a half hours. There was no cirrhosis in this phase.

Phase II extends from the sixth to the eighth week. In this phase, average ascorbic acid values 60 hours after the last injection were significantly lower than the average normal. The stress could still deplete the ascorbic acid and sudanophilia to practically the same extent as in Phase I. In this phase there was evidence of connective tissue proliferation, and on follow-up there was almost complete reversibility of the cirrhotic process.

Phase III extends from the 9th to the 11th week. In this phase the average ascorbic acid values 60 hours after the last injection were still lower than the average normal. However, the various stresses caused significantly lower depletions. The lowering of depletability was most marked in the histamine experiments, although there was also significant reduction in the corticotropin group. This phase was associated with irreversible cirrhosis.

Phase IV extends from the 12th to the 14th week. In this phase the average ascorbic acid values were normal, but the depletability continued to be lower. In the corticotropin experiment there was, however, a marked trend toward restoration of depletability in later stages. This phase was

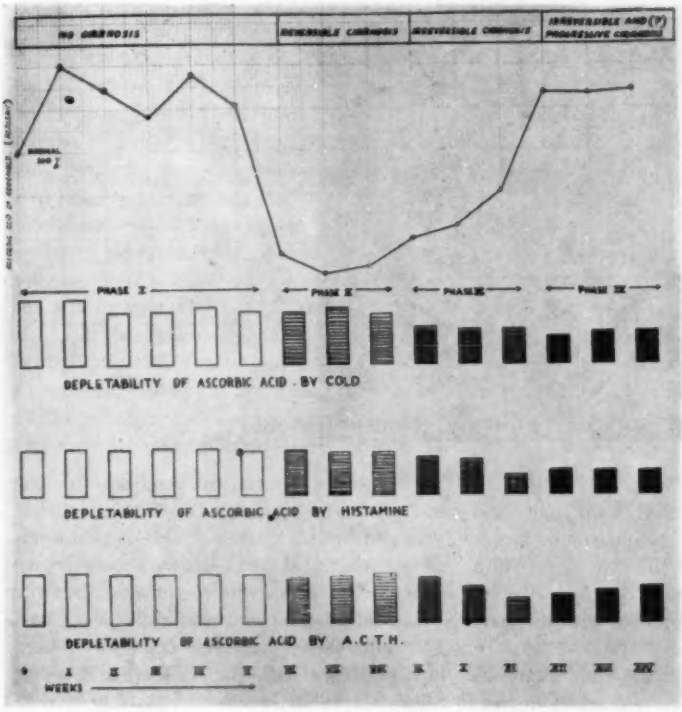

Chart 2.—Graphic representation of ascorbic acid values of the adrenal and their depletability by acid, histamine, and corticotropin in the four phases of the cirrhotic process.

associated with irreversible and progressive cirrhosis.

Histological and Histochemical Studies of the Adrenals

The hematoxylin-eosin stains were studied for the hypertrophy of the adrenal cortex and the ratio of the thickness of the zona reticularis to that of the zona fasciculata. In normal rats the ratio is roughly 1:1 (Fig. 12). In the animals of the first to the fifth week and in follow-ups of the subsequent weeks the adrenals showed a marked cortical hypertrophy, and this ratio

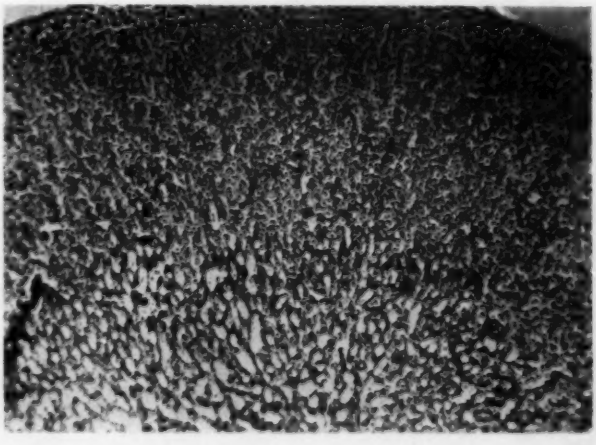

Fig. 12.—Normal rat adrenal: zona fasciculata-zona reticularis ratio is 1:1. Hematoxylin-eosin stain; × 80

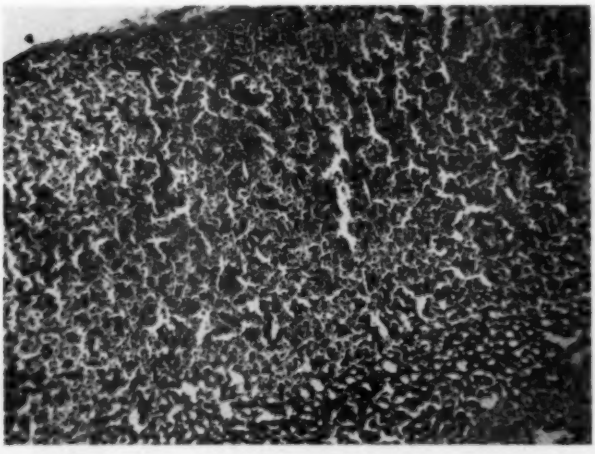

Fig. 13. — Fourth week. Rat adrenal: zona fasciculata-zona reticularis ratio is 3:1 Hematoxylin-eosin stain; × 80.

was altered to as much as 1:3 (Fig. 13). This was also associated with increased sudanophilia and clear cytoplasm in larger cells. Roughly, during the 6th to the 11th week there were only moderate hypertrophy and depletion of sudanophilia, with the zona reticularis-zona fasciculata ratio 1:1.5. In one case proliferation of the zona glomerulosa was also present to a marked degree. Necrotic or hemorrhagic lesions were conspicuous by their absence throughout the study. As could be seen in the preceding observations, the histological picture of the adrenal was well correlated with the ascorbic acid values (Table 2), higher ones being associated with hypertrophy of the adrenal cortex, involving chiefly the zona fasciculata. The 17-ketosteroids study did not prove fruitful, as the color contrast in normal, as well as injected, animals was not conclusive.

Comment

Our observations on normal rats exposed to cold stress, as well as to single injections of carbon tetrachloride, conformed to the observations of Sayers.[18] Both these stresses lead to depletion of ascorbic acid levels (Chart 1). We have taken this to mean activation of adrenal cortex, whereby ascorbic acid and sudanophilic material are utilized for elaboration of corticoids, which are required to meet the increased demands of the tissues in states of stress. Study of subsequent levels of ascorbic acid after stress shows return of ascorbic acid levels to normal, followed by an increase beyond normal levels (Chart 1). This later phase has been interpreted in accordance with Sayers[18] as a stage of relative adrenal inactivity, possibly due to the fact that during all the antecedent period sufficient corticoids have been produced and continued effect of corticotropin from the anterior pituitary has led to hypertrophy of secretory units. These become filled with material which can be utilized for subsequent stresses, if any, as shown by significant depletion of ascorbic acid at the 60-hour period, produced by exposure to cold stress of three and a half hours, as well as by injection of 0.1 cc. carbon tetrachloride (Chart 1).

The higher levels of ascorbic acid found in Phase I (Table 2), i. e., up to the fifth week, possibly imply that the adrenals were capable of recovering from the effects of injected carbon tetrachloride after discharging corticoids for meeting the stress. These high levels, however, belong to adrenals capable of being activated, as shown by the appreciable depletability of ascorbic acid on exposure to various stresses (Chart 2). The livers of these rats showed only

slight changes in reticulum or equivocal fibroplasia.

In Phase II (Table 2), i.e., from the sixth to the eighth week, adrenal ascorbic acid levels were low. This would mean active adrenals, perhaps implying that, even in spite of continued adrenal activity, stress had not been overcome, and that the output of steroids in the preceding 60 hours had not been commensurate with the degree of stress. Near-normal depletability of ascorbic acid by various types of stress (Chart 2) denoted that the adrenals had practically normal reactivity. In these cases, some fibroplasia, chiefly cellular, had taken place. Histologically, there were reversibility of cirrhosis and almost complete reconstitution of lobules.

In Phase III (Table 2) ascorbic acid values were either slightly low or normal. However, the depletability of ascorbic acid under cold stress was decreased (Chart 2). Low or approximately normal ascorbic acid values imply, as in Phase II, that the adrenals, even in spite of continued activity, had not yet emerged from the effects of stress and had not in 60 hours elaborated sufficient corticoids to overcome this. The decreased depletability implies that something probably had occurred which diminished the capacity of adrenals to enhance significantly their output of corticoids, in the face of new or increased stress. This phase was predominantly associated with irreversible cirrhosis.

In Phase IV (Table 2) higher values of ascorbic acid at the 60-hour period with lowered depletability require an explanation (Chart 2). These findings might mean one or more of three things: 1. Carbon tetrachloride has stopped acting as an effective stresser. That such a possibility exists has been suggested by workers, e.g., Stowell and associates,[20] and Cameron and Karunarantae,[6] who found that effects of carbon tetrachloride on liver cells and on subcutaneous tissue were progressively less marked with the passage of time. 2. Some block has occurred in the chain that normally is utilized for eliciting the general adaptation syndrome, e.g., at the level of the pituitary. 3. The adrenals have become nonresponsive to the humoral stimuli elaborated from the anterior pituitary.

Studies on the depletability of ascorbic acid under the effects of histamine and corticotropin provide some clue to this. Histamine acts as a stresser by activating the pituitary and causing it to release corticotropin. Corticotropin, on the other hand, acts without intervention of the pituitary on the adrenals. A perusal of the depletability under these two agents reveals that there is a progressive diminution of depletability of ascorbic acid from the 9th to the 11th week. However, at all these stages reduction is slightly more marked in the histamine group than in the corticotropin group (Table 2). In the following two weeks, i.e., the 13th and 14th weeks, when depletability due to corticotropin tends to increase, there is much less improvement in the depletability due to histamine. These observations suggest that possibly there is some change both at the pituitary and at the adrenal cortex level. The studies of Klein and associates[13] have also indicated that responsiveness of adrenal cortex to exogenously administered corticotropin is impaired in various liver diseases. The changes at the pituitary level appear to be more marked and persistent. It is of particular note that rapid change of reversible cirrhosis to the irreversible and progressive form at about the 12th week is associated with minimum depletability.

The cause of these peculiar developments can at best be speculative at this stage. Carbon tetrachloride acts as a stresser and elicits the general adaptation syndrome, through the liver cells, or other tissues of the body, such as the subcutaneous tissues, kidneys, etc., which stimulate the pituitary. The injury on the liver results in the production of certain factors locally, either at the liver cells or in the connective tissue elements which induce the proliferation of the latter. This proliferation seems to be

counteracted, in the early stages at least, by certain factors, one of them being the release of adrenal corticoids, produced as a result of the stress. The glucocorticoids are known to have such an effect. The exact site of this counteraction may be damaged cells, proliferating precursors of fibroblasts, or new blood vessels. This state in Phase II is still recovered from if further injections are discontinued. During this stage, when liver function is not much altered, the hormones liberated from the endocrines are probably successfully dealt with by the liver cells, and, consequently, their inhibitory effect on the pituitary is not significant. These possibilities have been represented in Chart 3.

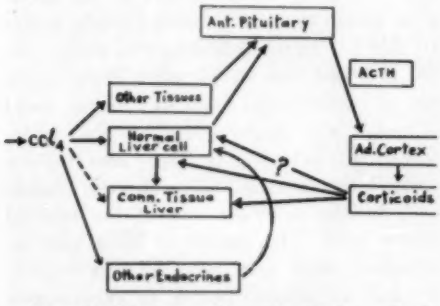

Chart 3.—Diagrammatic representation of the various probable interreacting factors in the reversible stage of cirrhosis.

If the injections are continued for long, carbon tetrachloride excites the general adaptation syndrome less and less effectively. This may in considerable measure be due to the metabolic peculiarities of the liver cell itself, which has a wide reserve and hence is endowed with the capacity of autonomous adaptation to the hepatotoxic situation, apart from the general adaptation syndrome. Similarly, the other tissues which act as mediators of the stress may acquire adaptation. Various alterations in the lobular architecture may introduce an as yet unknown and uncomputed factor which may be modifying the hepatic responses.

There is one other possibility. Cirrhosis is known to affect the various endocrine functions, possibly because of the altered capacity of liver cells to deal with the hormones. These substances tend to collect disproportionately in the body. Such an accumulation would act back on the anterior pituitary and hamper its capacity to release or elaborate the various tropic principles. Moreover, carbon tetrachloride may be toxic to the pituitary itself. Studies of Brown and associates [5] have shown that the rate of disappearance of hydrocortisone is inversely proportional to the degree of liver damage. They have inferred that there might be a homeostatic mechanism which results in decreased adrenocortical secretion in subjects with liver disease. The higher ascorbic acid values of Phase IV indicate not so much the recovery of adrenal function as its relative nonparticipation. Owing to the poor response to the stress and paucity of adrenal corticoids, the proliferation of connective tissue elements continues unabated and in due course it becomes irreversible. These possibilities have been represented in Chart 4.

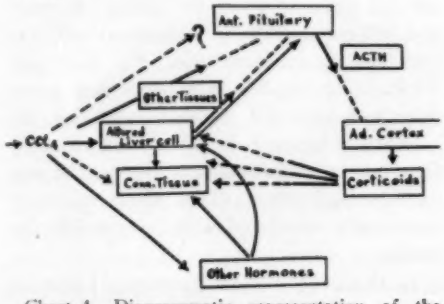

Chart 4.—Diagrammatic representation of the probable various interreacting factors in the irreversible stage of cirrhosis.

The above observations tend to indicate that noncirrhotic histology of the liver coexists with that state of adrenal function in which the adrenals are capable of recovering from the effects of stress at the 60-hour period. Reversibility of cirrhosis coexists with Phase II. In Phase III, when insufficiency of the adrenals, despite continuous activity and incapacity to further augment its function under new and increased stress, declares itself, irreversibility

of cirrhosis always slowly sets in. In Phase IV cirrhosis is decidedly irreversible, and even progressive, and coexists with diminished adrenal function, as well as with the diminished capacity of the adrenal to enhance its activity, due to adrenal and extra-adrenal factors.

The foregoing observations reveal that only highly cellular fibroplasia of the early stages associated with mild reticular proliferation is appreciably reversible. It has been postulated that during the process of laying down of fibrous tissue there are progressive changes in the ground substance, as well as collagen, such as the type of change from precollagen to collagen (Orekhovitch ‡) or the changes envisioned by Gersh and Catchpole.[11] Balasubrahmanyan[3] has found no correlation of irreversibility with histochemically demonstrable changes in the ground substance. It is possible, then, that changes in polymerization of collagen or other changes, loosely referred to as maturation, which develop simultaneously with, and probably because of, alterations in adrenal function, change it into an irreversible form.

Summary

It appears from this study that the adrenals become variously involved in the chain of events started by carbon tetrachloride. The degree and nature of their involvement probably condition the picture of liver that emerges at the various phases of the chronic carbon-tetrachloride injury. The reversible stage of cirrhosis is associated with a normally functioning adrenal, or at least one that can be induced to further activity when subjected to stress. With the onset of irreversibility, the adrenal cortex is noted to be functionally damaged. The mechanism of this variability is briefly discussed.

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Adrenal Cortex and Hepatic Cirrhosis

II. Effect of Cortisone on Progress of Carbon-Tetrachloride-Induced Cirrhosis

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Studies on the effect of cortisone and corticotropin on cirrhosis of the liver have in recent years attracted considerable attention, both of experimental workers and of clinicians. The results of these studies are, however, conflicting. Aterman [1] and Cavallero and associates [5] noted a reduction in the fibrous tissue content of the livers of rats if cortisone was given simultaneously with carbon tetrachloride. Diengott and Ungar,[6] on the other hand, observed that cortisone therapy may actually aggravate the cirrhosis thus induced. Aterman and Ahmad [2] reported considerable deterioration of liver function in such rats.

Bluemle [3] and Brown [4] and associates, in their clinical studies, noted reduction in fibrosis and fatty infiltration after corticotropin. Helm [7] reported marked clinical improvement with cortisone in a case of decompensated cirrhosis, and Zoeckler and Hezstrum,[14] similarly, concluded that cortisone had a definite place in the treatment of the disease. Zoeckler,[13] in a later controlled study, however, made the observation that the addition of cortisone to the regimen of cirrhotic patients resulted in a beneficial effect upon the plasma protein concentration but there was no effect on the other liver functions or on the hepatic architecture. No fatty metamorphosis was noted. William and Flink [12] similarly tried corticotropin on 10 patients of chronic liver disease and were doubtful whether any patient benefited significantly. Rich [8] and Steinberg [10] and their associates observed fatty change in the liver with corticotropin, although Selye [9] denied this effect.

Most of the clinical studies have been done on advanced cases, and no attempt has been made to study the effect of cortisone in the reversal of early fibrosis. In experimental studies, also, cortisone has been administered simultaneously with the cirrhogenic agent.

In the present investigation, which is a corollary of Part I of this study,[11] an attempt was made to determine the effect of cortisone on the progress of cirrhosis after the injections of the cirrhogenic agent had been stopped.

Materials and Methods

Young albino rats of both sexes were given semiweekly injections of carbon tetrachloride. The progression of cirrhotic changes was studied by killing them in batches at weekly intervals, and the regression of cirrhosis was observed by setting aside another batch simultaneously which were spared further injections and were killed after two and three weeks, as described earlier.[11]

From the eighth week on a third batch, of six rats, was isolated every week; these were similarly spared further injections of carbon tetrachloride and were given, instead, daily intramuscular injections of cortisone acetate in the following regimen: 5 mg. per day per rat for six days, 10 mg. per day per rat for three days, and 5 mg. per day per rat for two days. A further period of three days was allowed to elapse before this set of animals was also killed,

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on the same day as the two-week follow-up which served as control. Histological and collagen content studies of the liver were carried on, as reported previously.[11]

Observations

The histological changes and collagen content of the livers in the "forward" and follow-up groups have been discussed in detail earlier.[11]

The effect of cortisone was studied only when the fibrosis in the liver had well set in and after the injections of carbon tetrachloride had been stopped. As is apparent from the accompanying Table, it was only in the eighth- to ninth-week period that cortisone could be noted to have an ameliorating effect on the cirrhotic process. The first follow-ups in this instance acted as control, as both this batch and the cortisone batch from the same group of rats were killed on the same day. In the eighth week, the fibrosis in the cortisone batch was of distinctly less severity and the livers were closer to normal than those of the first follow-ups. The difference became less and less marked from the 10th week on. The cortisone-treated animals continued to show a severe degree of pseudolobulation and fibrosis. There was, however, an equivocal thinning of the argyrophilic bands in these animals as compared with the controls.

Correlation of Average Grades of Fibrosis in the Cortisone and Control Groups

Weeks	Cortisone Group		Control Group	
	Fibrosis (Average Pluses)	Collagen Mg/gm.	Fibrosis (Average Pluses)	Collagen Mg/gm.
8	1.4 (1–2)	5.31 (3.38–7.32)	2.3 (2–3)	6.32 (5.12–7.31)
9	2.3 (1–4)	7.94 (3.35–10.25)	2.5 (2–3)	8.3 (4.19–12.71)
10	3.4 (1–5)	16.06 (8.65–25.75)	4.5 (4–5)	20.97 (15.97–26.05)
11	4.4 (4–5)	19.67 (15.37–28.88)	4.2 (3–5)	21.15 (11.38–27.41)
12	4.2 (4–5)	21.46 (18.27–29.74)	5 (5–5)	25.6 (21.37–28.39)
13	4.8 (4–5)	25 (18.03–29.67)	5 (5–5)	27.53 (26.3–28.76)
14	4.75 (4–5)	32.75 (26.84–36.25)	4.5 (4–5)	30.74 (26.59–34.89)

In the collagen studies also it was noted that fibrosis regressed only up to the eighth week in the cortisone group. From the ninth week on the collagen content was about the same as that of the second follow-up group.

One thing was thus evident; i.e., there was no enhancing effect of cortisone on the cirrhotic process at any stage. Cortisone helped to ameliorate the cirrhosis and accelerate the reversal when the process was in the reversible phase, but once cirrhosis had well set in, it was totally ineffective.

Comment

It has thus been observed that cortisone helps in rapid regression of the cirrhotic process at the eight-week stage, when even otherwise the cirrhosis is reversible. At a later stage, its effects are less and less marked, amounting to nothing more than slight thinning out of the reticulum bands. This observation is more in conformity with that of Aterman [1] and unlike that reported by Diengott and Ungar.[6] It has been stated earlier that the irreversibility of cirrhosis may be related to the changes in polymerization of collagen, like the change from precollagen to collagen (Orekhovitch *), which develop simultaneously with, and perhaps because of, the insufficiency of adrenal function. The thinning out of the connective tissue bands possibly implies a removal of newly laid collagen under the influence of cortisone, whereas the previously laid connective tissue fibers fail to regress. It has thus been shown that collagen has almost no metabolic turnover in vivo, as compared with the earlier elements of connective tissue. Cortisone seems only to accelerate the natural turnover but cannot possibly induce it where there is none. These results also tend to support the notion that hypoadrenocorticalism due to adrenal or extra-adrenal factors would favor deposition of fibrous tissue in the liver.

Contrary to some other reports,† no fatty

* Orekhovitch, V. N., cited by Aterman.[1]

† References 8 and 10.

change was observed. Parenchymal damage was, however, unmistakably severer in the animals receiving cortisone. This was manifested by a depletion of the cytoplasmic nucleoproteins. This is understandable, as cortisone is an antianabolic hormone, and hence, particularly in the presence of a necrotizing agent, it interferes with the protein synthesis.

Summary

This study indicates that cortisone helps in the regression of cirrhosis during the phase when the process is naturally reversible. At a later stage no appreciable regression is caused. It is felt that cortisone helps to undo only certain earlier changes in the maturation of collagen. Once mature fibrous tissue is laid down, cortisone is ineffective.

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POTENTIAL ROLE OF NON-NUTRITIVE FOOD ADDITIVES AND CONTAMINANTS AS ENVIRONMENTAL CARCINOGENS

(Special Article)

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A RAPIDLY growing number and variety of non-nutritive substances have been introduced during recent decades into foodstuffs intended for general human consumption through the use of modern methods of food production and processing. Some of these chemicals are intentionally added to foods for various reasons, whereas others are employed for different purposes in the production of foodstuffs and remain unintentionally in them as residues. A disturbing aspect of this development is that there exists no mandatory provision for assuring, a priori, that biologic properties of each of these additives and contaminants, particularly later long-term or delayed effects, have adequately been studied. The circumstances suggest the virtual certainty that many have not. It is especially important in this respect that observations made during recent years in men and experimental animals have demonstrated a not inconsiderable number of chemicals similar to, or identical with, those introduced into foodstuffs which possess carcinogenic properties. The actual or possible existence of cancer hazards related to carcinogens in foodstuffs therefore poses a serious public health problem, since the daily and lifelong exposure to such agents would represent one of the most important of the various potential sources of contact with environmental carcinogens for the population at large, acting on both the healthy and the sick, the metabolically normal and abnormal alike. Protection of a sufficient and, at the same time, safe food supply is of general concern and deserves the attention not only of the food-producing, processing, and merchandizing industries and their industrial suppliers but also of all other parts of human socicty, such as various general and local governmental agencies, labor and industrial management organizations, insurance companies, the medical profession, and, above all, the general public. This problem has, moreover, a certain international importance because of the importation of raw and processed foodstuffs from countries of production into countries of consumption.

1. A Panoramic View of Food Additives and Contaminants

A listing of the main groups of food additives and contaminants which are intentionally or unintentionally introduced into foodstuffs to be used for human consumption includes a large number of highly diverse chemicals and provides an illustration of the scope and type of problem to be dealt with.

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National Cancer Institute, National Institutes of Health, U. S. Public Health Service, Department of Health, Education, & Welfare.

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A. Food Additives
   1. Natural and synthetic dyes
   2. Antioxidants of fats and lipids and vegetable matter
      (*a*) Destroyers of peroxides formed during auto-oxidation
      (*b*) Oxygen acceptors
      (*c*) Hydrogen donators
   3. Natural and synthetic mucilages, thickeners, and gelatinous materials
   4. Synthetic sweeteners
   5. Synthetic flavoring agents
   6. Surfactants (detergents, foaming agents)
   7. Humectants
   8. Preservatives and chemical sterilizing agents
   9. Water conditioners (iodine, fluorides)
   10. Antifoaming agents
   11. Salt substitutes
   12. Shortenings
   13. Antistaling agents and softeners
   14. Bleaches
   15. Food modifiers and improvers (meat tenderizers, etc.)
   16. Oil and fat substitutes of petroleum derivation
   17. Organic solvents used as vehicles of some additives
   18. Hydrogenated oils and fats (containing saturated, instead of the biologically important unsaturated, fatty acids and, possibly, nickel as a contaminant)
   19. Hygroscopic and antihygroscopic agents
   20. Emulsifiers and solidifiers

B. Food Contaminants
   1. Pesticide residues
      (*a*) Bactericides
      (*b*) Insecticides—miticides
      (*c*) Rodenticides
      (*d*) Molluscicides
      (*e*) Fungicides
      (*f*) Herbicides
      (*g*) Nematocides
      (*h*) Defoliants
   2. Antisprouting and antimaturation agents of fruits and vegetables
   3. Insect repellents
   4. Hormonal fattening agents—estrogens
   5. Antibiotics (fed to food animals and added to foodstuffs)
   6. Antienzymatics
   7. Enzymes
   8. Pan glazes (silicones)
   9. Pan greases (mineral oils)
   10. Water pollutants: coal tars and oils, petroleum tars, asphalts, oils, refinery and coke-oven effluents, chromates, radioactive substances, arsenicals, etc.
   11. Chemical sterilizing agents
   12. Wrapping and coating materials (paraffin, waxes, resins, plastics)
   13. Soot adherent to smoked foodstuffs and roasted and toasted products
   14. Household detergents and their coloring agents (stilbene derivatives)
   15. Non-ionizing radiation (ultraviolet) products
   16. Ionizing radiation (radioactive) products
   17. Radioactive substances taken up by plants and food animals from contaminated soil or water or adhering to them in the form of radioactive fall-out

The extent to which foodstuffs contain additives and contaminants is evident from the facts that there is a list compiled by American and English investigators of some 800 to 1000 food additives and that many foods daily consumed by the general public are contaminated with pesticide residues of some type, such as arsenicals or chlorinated hydrocarbons (D.D.T., etc.). Only very fragmentary

information is available as to the total amount of food additives annually consumed in different countries. There exists, moreover, a constant change in the exposure of the consuming public to the various additives and contaminants because of the introduction of new chemicals for this purpose, replacing, in part, some formerly used. This development is particularly prominent in the field of pesticides.

The average annual production of arsenical insecticides (lead arsenate, calcium arsenate) in the United States, which stood during 1941 to 1944 at almost 70,000 short tons, had dropped to 16,500 short tons in 1949. Synthetic organic insecticides, briefly mentioned without citing production figures in the 1939 edition of "Synthetic Organic Chemicals, United States Production and Sales," were discussed in the 1953 edition in detail in a separate chapter. The 1953 production of the United States of both cyclic and acyclic insecticides amounted to 192,000,000 lb.; of these, 84,000,000 lb. represented the output of D.D.T. A total of 356,000,000 lb. of all types of pesticides was produced in 1953 in the United States.

The production and consumption of detergents and antibiotics furnish an additional illustration of the changing panorama of exposure conditions to which the general public is subjected in connection with food additives and contaminants. Neither one of the two groups of chemicals is mentioned as a distinct item in the 1939 edition of the report of the U. S. Tariff Commission on "Synthetic Organic Chemicals, United States Production and Sales." In the 1953 edition of these annual reports, the production figure of surface-active agents is given as 921,594,000 lb., of which 364,000,000 lb. were in detergents of the dodecyl-benzene-sulfonic-acid type, 129,000,000 lb. were in sulfonated petroleum aromatic compounds, 127,000,000 lb. were in sulfated and sulfonated alcohols, and 327,505,000 lb. were of the acyclic variety (salts of fatty acids). The production of antibiotics for animal feed supplements during 1953 stood at 434,000 lb., as compared with 258,000 lb. in 1952. During the same year 4000 lb. of diethylstilbestrol were sold, the bulk being used for speeding up the fattening of fowl and livestock.

Polymerized macromolecular synthetic chemicals, such as polyethylene, cellophane, and polyvinyl chloride, have found during recent years extensive and rapidly rising use in the production of containers, coating of cans, and wrapping and coating materials of foodstuffs, replacing in part paraffinized packaging materials, and have been used also in foods as substitutes of gelatin and as emulsifiers and stabilizing agents.

With the growing practice of consuming commercially preserved and packaged foodstuffs, the addition of synthetic food dyes has become increasingly common and has spread to a rising number and variety of fresh and processed foods. Whereas the total amount of synthetic primary coal tar dyes certified by the Food & Drug Administration, Department of Health, Education, and Welfare, stood at almost 1,150,000 lb. in 1948, it had risen in 1954 and 1955 to over 1,500,000 lb. annually. The same trend is apparent from data on the spreading use of food dyes in Israel. The total amount of food dyes imported into this country in 1953 stood at 580 kg., had risen in 1954 to 2766 kg. and was 2202 kg. for the first seven months of 1955. The quantity of food dyes tested and approved by the National Hygienic Laboratory of Japan amounted to 13,260 kg. in 1950, stood at 12,900 kg. in 1952, and rose to 28,020 kg. in 1954. The total

amount of food dyes used in France during 1953 was about 42,000 kg. The average annual importation of food dyes into Australia during the period 1950 to 1954 amounted to approximately 70,000 lb.

Apart from the apparent considerable differences in the per capita consumption of food dyes in various countries, an additional variation in exposure conditions is introduced by marked national discrepancies in the number and type of food dyes used. The report of the Pharmacology Panel of the Food Standards Committee, Report on Colouring Matters, of Great Britain, 1954, listed 79 different colors which have been used extensively in foods and beverages in several countries. Mono-, di-, tri-, or polyazo dyes contributed a total of 36 colors. Triphenylmethane dyes furnished 18 colors; there were also 6 xanthene dyes, 3 pyrazolone dyes, 3 azine dyes, and 1 each of the class of nitro- and diphenylmethane and indigoid dyes. In view of the fact that different countries have considerable variations in the type of purity standards and control measures observed, and that many food dyes, including certified ones, are neither chemical entities nor free from appreciable amounts of impurities, additional factors of regional variations in exposure conditions for populations of different countries are introduced. These are intensified by the considerable differences in the number of dyes employed for coloring foodstuffs. While only 5 synthetic dyes are permitted for this purpose in India, their number stands at present at 15 * in the United States, at 17 in Turkey, at 24 in Japan, at 26 in Norway, at 27 in Sweden and New Zealand, at 28 in South Africa and Spain, and at 32 in Finland. A possibly much larger number of dyes may be introduced into foodstuffs in those countries which have only a list of proscribed dyes. It may be noted that some dyes which are on the permitted list in some countries are included among the prohibited dyes in other countries.

It is obvious from these few illustrations of the type and extent of human exposure to food additives and contaminants, that economic, climatic, industrial, and other factors make for marked regional variations in contact of the general population with these alimentary ingredients. Health and cancer hazards which might be associated with such agents, therefore, may vary for these reasons in different countries and population groups. An additional complicating factor in assessing the presence and type of health and cancer hazards related to these factors may be provided by the probability that entirely new, or synergistic or antagonistic, effects upon the human organism may result from the synchronic or heterochronic action and interaction of the multitude of additives and contaminants before and after their entering the body of the general consumer.

2. Carcinogenic Aspects

The rapidly growing interest in the apparent role which environmental physical and chemical agents play in the production of human cancer has focused attention upon possible cancer hazards related to the large number of food additives and contaminants. The rising appreciation and awareness of such possible sources of human carcinogenesis has found expression during recent years in numerous articles appearing in the scientific and lay press and in trade journals, as well as

* The further use of the following three food dyes was prohibited during the latter part of 1955: FD&C Orange I; FD&C Orange II; and FD&C Red 32, reducing thereby the number of certified food dyes from 18 to 15.

in proceedings of legislative committees and comments made through radio and television channels.

The presently existing unsatisfactory situation in these matters is attributable to several factors. The bulk of the still rather restricted pertinent information on potential cancer hazards from food additives and contaminants is of relatively recent date. Knowledge of such observations is often limited to parties mainly interested in scientific aspects of carcinogenesis and is sometimes not fully appreciated by those parties concerned with the practical aspects of potential human cancer hazards inferable from these experimental findings. There exists, moreover, a definite degree of uncertainty as to the actual applicability to the human consumer of some of the results obtained in experimental animals by the administration of several food additives and contaminants. Since the existing methods of testing chemical agents for potential carcinogenic properties are technically difficult, time-consuming, and expensive and require experienced and competent personnel for their performance, their large-scale and routine application to food additives and contaminants has met some resistance, for various reasons. In the light of these and other circumstances there can be no doubt that many of these agents have not adequately been investigated for carcinogenic qualities.

Epidemiologic human evidence provides adequate proof that a prolonged dietary intake of drinking water, wine, and other foodstuffs contaminated with arsenicals from industrial sources or in the form of insecticide residues has caused cancer of the skin in some of the exposed persons. Observations made on man and/or experimental animals provide conclusive evidence concerning a carcinogenic potency of coal tars, various petroleum derivatives, and radioactive matter, although human evidence is lacking regarding such effects if these agents are present as pollutants of drinking water, or of foodstuffs in the processing or preparation of which contaminated water was used. The prolonged ingestion of radioactive water for medicinal purposes, however, has given rise to the development of sarcomas in several persons.

The carcinogenic action of natural or synthetic estrogens, used as fattening agents for poultry and livestock by the food industry, when given to experimental animals, is well established. Such an action is suspected when estrogens are administered for medicinal reasons to man or are produced by certain types of estrogen-producing ovarian tumors. Prolonged feeding to rats of large amounts of D.D.T., thioacetamide, and thiourea, extensively employed as pesticides, resulted in the development of hepatomas. This tumorigenic action of D.D.T. was said to be minimal when amounts of the chemical were fed which are much higher than those encountered under ordinary conditions of human exposure. An appreciable hepatoma-producing action recently was demonstrated in rats fed a new commercially available miticide (Aramite 2-[*p-tert*-butylphenoxy]-1-ethylmethyl-2-chloroethyl sulfite). While the use of this pesticide was permitted, its maximal permissible content in foodstuffs being set at 1 part per million (hepatogenic at a level of 500-5000 parts per million), the use of thiourea and thioacetamide as fungicides was prohibited in the United States on the basis of the experimental evidence. The same fate was shared by Dulcin (*p*-ethoxyphenyl urea), a synthetic sweetening agent, which, when given to rats by the oral route, elicited tumor formation in the liver, according to one investigation. This result, however, has not been confirmed by subsequent investigators.

The humectant diethylene glycol, when fed to rats, caused the development

of benign and malignant bladder tumors (papillomas and carcinomas). Dodecyl benzene, when applied to the skin of mice, proved to have a co-carcinogenic action and a doubtful carcinogenic one. Polyvinyl alcohol, crosslinked by exposure to formaldehyde, elicited, when subcutaneously implanted in rats, the development of sarcomas. Similar sarcomatous effects were obtained in rats and mice with subcutaneous or intraperitoneal implants of small pieces of various plastic films (cellophane, polyethylene, polyvinyl chloride, etc.). The tumors developed at the site of deposition of the macromolecular polymers.

Among the various formerly and presently used synthetic food dyes, carcinogenic properties were discovered during recent years in a surprisingly large number when tested in rats and mice. Malignant tumors of the liver and bladder have been produced in rats and mice given derivatives of *p*-aminoazobenzene, of which butter yellow, or *p*-dimethylaminoazobenzene, is a formerly widely used food dye. Neoplastic reactions in the liver resulted in rats and mice only when either overwhelmingly large doses of these azo compounds were administered with the food or when the animals were kept on a riboflavin-deficient diet. When fed to dogs, o-aminoazotoluene or *p*-dimethylaminoazobenzene, elicited benign and malignant bladder tumors. Hepatomas also developed in mice after an oral introduction of oil orange E (benzene-azo-β-naphthol), while another food dye, oil yellow HA, may have similar properties, since its oral administration was followed by the appearance of a hepatoma in one mouse and its subcutaneous introduction resulted in the development of a spindle-cell sarcoma. Oil orange TX (1-[2-tolylazo]-2-naphthol), when injected subcutaneously into mice, elicited spindle-cell sarcomas at the site of injection, as well as adenocarcinomas of the ileocecal junction. The oral administration of yellow AB (1-phenylazo-β-naphthylamine) and yellow OB (1-*o*-tolylazo-β-naphthylamine) fed to rats at a level of 2.5% was followed by the development of liver cancer in a few animals. These observations with food dyes of the azo type deserve serious consideration because of complementary findings made in man and experimental animals regarding the carcinogenic action of closely related aromatic amino compounds, such as β-naphthylamine, benzidine, and 4-amino-diphenyl, which have elicited bladder cancers in man and bladder cancers and/or cancers of the intestinal tract in dogs and/or rats, and which possess a very high carcinogenic potency. Since several of the dyes listed are derivatives of β-naphthylamine or of 1-amino-2-naphthol, the potential urinary metabolite of α-naphthylamine, the possibility exists that these carcinogenic components of certain food dyes may be liberated in the body through a metabolic splitting of the dyes. The probability of such a metabolic degradation is suggested by spectrographic studies conducted on the urine of dogs and man given yellow AB by mouth. The urines of man and dogs contain under such conditions a substance similar to or identical with 2-amino-1-naphthol, the carcinogenic urinary metabolite of β-naphthylamine.

Several sulfonated triphenylmethane dyes, such as light green SF, brilliant blue FSF, and fast green FCF, elicited fibrosarcomas in rats at the site of subcutaneous injection, although tumors did not appear after their prolonged oral administration.

The production of papillomas and carcinomas of the forestomach in rats fed 4-nitrostilbene and of hepatomas and cancers of the Eustachian tube in rats given 4-aminostilbene orally is of importance because of the recent introduction of stilbene compounds as coloring matter in many household detergents used for

the cleaning of kitchen utensils, dishes, and cooking equipment of homes and commercial eating places.

Potential carcinogenic contaminants also may be introduced into foodstuffs if vegetables, fruits, fish, oysters, and livestock are grown on soil or in water polluted with known carcinogens, such as radioactive matter, arsenicals, selenium, and polycyclic hydrocarbons contained in ship fuel oils, since these chemicals may be taken up and stored by the vegetable and animal matter growing in such contaminated media. Consideration, moreover, must be given to the possibility that carcinogenic chemicals may be formed from noncarcinogenic ones under the influence of heat, resulting in the formation of charred or tarry carbonaceous matter, such as that formed when bread or biscuit is excessively toasted or meats are grilled or roasted, or when mineral oils used as fat substitutes are subjected to heat during grilling or baking of foodstuffs and, therefore, may be cracked and converted into carcinogenic hydrocarbons. There exists also the possibility that originally noncarcinogenic additives and contaminants may interreact with each other or with food constituents and form new compounds possessing carcinogenic properties in the foodstuffs. They may be produced under the influence of processing procedures or during the preparation of food in the kitchen. Plastics used as wrapping material, sausage skins, and coating material of fruits, cheese, meat, butter, and can linings may carry a similar hazard.

Mention may finally be made of several experimental observations indicating that a dietary intake of certain spices or alkaloids which may contaminate foodstuffs (chilies, alkaloids of Senecio plants, crude ergot) may result in the development of liver tumors (chilies, Senecio alkaloids) or neurofibromas (ergot) when given to rats.

The use of various types of radiating energy in the processing of foodstuffs also deserves consideration from a carcinogenic viewpoint, since these agents (ultraviolet radiation, ionizing radiation) produce in the constituents of food, such as sterols and nucleoproteins, definite chemical changes. No reliable information exists and no adequate experimental studies have been made to establish the noncarcinogenic nature of the radiation products, although both types of radiation are eminently carcinogenic when acting on living tissues, of both man and various species of animals.

Although the great majority of the different cancerous reactions elicited by the various food additives and contaminants mentioned were produced either by the administration of excessively high doses or followed upon their introduction through unphysiologic routes or routes distinct from those encountered under ordinary alimentation, and developed in animals differing to various degrees in metabolic respects from man, the mere fact of the existence of such responses, nevertheless, presents a definite warning deserving serious attention if possible endemic and epidemic cancerous manifestations among exposed population groups are to be avoided.

3. Objectives of a Control Program and Criteria of Cancer Hazards

Because of these observations incriminating certain food additives and contaminants in the production of cancers, and in view of the possibility that a comprehensive investigation of other such agents may result in the discovery of additional dietary carcinogens, the organization of a comprehensive investigative program on these matters appears to be desirable with the following objectives:

(*a*) To study the general and specific nature and scope of the problem and to collect the available evidence and to ascertain the defects in our present knowledge

(*b*) To devise technical means of procedure for studying and routine screening of food additives and contaminants, as well as of foodstuffs containing additives and contaminants before and after they have been subjected to various processes used in the preparation of food for carcinogenic or co-carcinogenic properties in experimental animals

(*c*) To develop reliable criteria for evaluating experimental evidence in terms of potential and actual human cancer hazards, and for creating thereby a rational basis for competent and fair decisions on the use of such agents in foodstuffs intended for human consumption

(*d*) To disseminate information to all interested parties concerning potential and actual cancer hazards from food additives and contaminants, the available methods of testing, and the interpretation of the results obtained with the screening procedures used.

During recent years several statements have been made by authoritative parties which may well serve as guiding principles in the general approach to the study and control of cancer hazards related to food additives and contaminants.

In discussing chemical carcinogens, Heller, Director of the National Cancer Institute, stated in 1950:

Considering that a danger of the chemical cancerigens lies in the slow, almost unnoticeable harm that comes from contact with them, it might be wise from a preventive point of view to consider all chemical agents which have elicited cancer in animals as having the potential properties for producing cancer in the human organism.

In its resolution passed at the Sixth International Cancer Congress held at São Paulo, Brazil, on July 26, 1954, the Committee on Cancer Prevention of the Commission on Cancer Control, International Union Against Cancer, expressed a similar sentiment:

In the case of agents whose carcinogenicity for man is not known but which elicit cancer in experiments conducted upon animals, although it is recognized that the development of cancer in response to such materials may be conditioned by the type of exposure, notably the species of animals or the route of administration, it is not prudent to regard such agents as harmless for man.

In view of the evidence that certain types of cancer result from exposure to agents that enter the body from the external environment, a basis for the prevention of these types of cancer is afforded by identification of sources of exposure to such agents and development of feasible measures for minimizing exposure of human population.

For properly appraising these statements in relation to cancer hazards from food additives and contaminants, the following definitions may be appropriate:

Chemical Additive: A chemical used as an intentional or adventitious addition to the basic foodstuff or produced in the basic foodstuff under the influence of an external physical agent (heat, vibration, ultraviolet radiation, ionizing radiation, ultrasonic radiation, electric radiation, etc.) during the production, processing, or storage of a food and present in the food when purchased. The additive may or may not have any nutritional value and may or may not alter the nutritional value of the basic food to which it is added.

(*a*) Intentional chemical additive: A chemical used in the manufacture of formulation of a food for the purpose of imparting some desired quality to the food or of serving a functional purpose in the food.

(*b*) Incidental chemical additive (contaminant): A chemical which is not required in the final food product but which is present as a result of having been used in the production, processing, or storage of the food.

Cancer Hazard: A reasonable possibility or probability that cancer may develop from contact with an agent intentionally or incidentally present in food.

Carcinogen: A chemical, physical, or animate agent which is capable of producing cancer in any organ or tissue of any species following exposure to it in any dose and physicochemical state and when given by any route, either once or repeatedly.

Co-carcinogen: A chemical which has a specific accentuating action in a sort of catalytic capacity upon the carcinogenic action of a carcinogen; i. e., it boosts and accelerates the action of the carcinogen so that ordinarily ineffective exposures to it may result in cancerous development. The action of such co-carcinogens not only is carcinogen-specific but seems also to be tissue-specific.

The action of co-carcinogens has to be distinguished from a nonspecific effect

of procarcinogens or anticarcinogens, which merely facilitate or hinder the relative effectiveness of a carcinogen upon tissues; i. e., they influence permeability of tissues, mucous membranes, and cells to carcinogens or the contact with living matter or favor the preservation or destruction of carcinogens or their retention or removal from tissues. Solvents or vehicles of carcinogens sometimes have such effects.

The potential importance of the ingredients present in the various vehicles in which the active principles of intentional and incidental chemical additives are often contained deserves serious consideration in carcinogenic respects, since some of them possess by themselves carcinogenic or co-carcinogenic properties (methylated naphthalenes, dodecyl benzene), whereas others used as solvents (alcohols, glycols, ethers, etc.), dispersing agents and activators (detergents), anticlumping agents, diluents, emulsion stabilizers, stickers, spreaders, attractants, and softeners have not as yet been tested, although they are often designated on labels as "inert" matter.

There is no necessary relation between toxicity and carcinogenicity of chemical agents. Since the appearance of cancer usually requires a long preparatory period, precautionary standards of testing suitable for ascertaining chronic toxic effects are inadequate in many instances for determining cancerigenic properties of a particular chemical. In fact, only occasionally have chronic toxicity tests in the screening of chemicals for toxic qualities revealed their carcinogenic character (2-acetylaminofluorene, originally intended as an insecticide; 430-styryl, developed as a trypanocidal agent), because they were carcinogenically highly potent. As a rule, the minimal carcinogenic dose is distinctly lower than the minimal chronic toxic dose. It is for this reason that not infrequently carcinogenic reactions may develop upon exposure to carcinogenic chemicals without a preceding or simultaneous appearance of any toxic symptoms.

4. Considerations and Design of Experimental Procedures Used in Carcinogenicity Tests

Exogenous carcinogens, like pathogenic micro-organisms, are eminently species-specific; some are even tissue- and strain-specific. The only universal carcinogen is apparently ionizing radiation. The target organ in which cancers may develop upon contact with environmental carcinogens depends upon the organ or tissue of primary contact, upon the relative solubility in biologic fluids, and therefore on the speed of removal from the site of primary contact of a carcinogen, upon the metabolism of the carcinogen, on its route of excretion, and on the tissue or organ of retention or deposition of a carcinogen. The target organ may vary in different species. It seems that not only the species specificity of a carcinogen but also the target organ depends upon species-specific metabolic processes. This factor apparently influences also the species-specific length of the latent period, which may be short for one species and long for another for the same carcinogen, and is unrelated to the average life span of a species. The relative length of the preparatory period also depends upon the potency of a carcinogen and upon the intensity of contact with it; i. e., the length of latent period decreases with an increasing potency of a carcinogen and with an increasing intensity of exposure to it. The degree of susceptibility to a carcinogen is also influenced by inherited, congenital, or acquired constitutional factors. Sex-related factors seem to play in man a minor role in this respect. The same con-

sideration applies to age. The tissues of the young are as susceptible as those of the old, if not more so. In fact, it is likely that at least some of the cancers observed at birth or in infants and children are attributable to an exposure of the maternal organism, before or during pregnancy or lactation, to carcinogenic agents which passed the placental barrier or were secreted in the milk.

In addition to the carcinogens proper, having direct or remote action, there exist agents producing apparently primary functional and/or anatomical organ or tissue alterations which, through the mechanism of normal or abnormal endogenous factors, often of hormonal nature, produce cancers in the affected organs or tissues or remote from them. The development of cancers of the sex organs on the basis of disturbances of the sex hormonal metabolism following upon chronic nonspecific liver injuries provides the best example of this type of indirect environmental carcinogenesis. Since many of the modern pesticides are definite hepatotoxins, the possibility of indirect carcinogenesis deserves consideration in the evaluation of food-produced cancer hazards.

In the assessment and testing of cancer hazards from food additives and contaminants it is also necessary to use animals with artificially produced disturbances of metabolically important functions (detoxication, excretion, metabolites, endocrinic products), such as those represented by diabetes mellitus, chronic nephritis, cirrhosis of liver, hypothyroidism, hyperestrinism, malnutrition, and abnormalities of the gastrointestinal function and flora. Such conditions not only may alter the degree and duration of retention and organ affinity of carcinogenic agents, as well as the rate of metabolic conversion, but also may produce changes in the type of metabolic pathways used and the character and relative amounts of metabolites generated.

In developing realistic and rational test conditions, consideration also must be given to the fact that, under the circumstances prevailing in human exposures, nutritional agents are subject to various influences which may change their chemical character—heating, interaction with other food ingredients, acids, alkalis, metal catalysts from cooking vessels, co-carcinogenic agents, and synergistic action of other constituents of foodstuffs (dyes, chlorinated hydrocarbon pesticides, hepatotoxic agents of various chemical character), as well as of occupational and general environmental agents (medicines, cosmetics, air pollutants, habits, hobby and sport factors, cleaning and polishing agents, sanitary goods, etc.). These considerations indicate that in carcinogenic tests the agents to be studied should not merely be added to prepared food, but should be incorporated into various foods, which then are subjected to preparatory conditions identical with those used for food intended for human consumption.

In experimental attempts to determine the presence of cancer hazards related to a food additive or contaminant, the experimental conditions to be used should give answers to two main questions: (*a*) Does the agent produce cancer in any species under any type of exposure (dose, route) and in any organ or tissue? (*b*) Does the agent elicit tumor formation in any species and in any organ or tissue when administered under conditions identical with or similar to those prevailing in human consumption?

Particularly thorough and extensive studies should be made of those agents which upon chronic toxicity studies proved to be mitotic poisons; produced ambivalent (aplastic and hyperplastic) reactions; elicited in the offspring congenital defects, abnormalities, or monstrosities; proved to be hepatotoxins or hemato-

toxins; caused distinct disturbances of endocrine functions, especially those of the pituitary gland, thyroid, adrenal, and sex glands; exhibited cross-linking properties, or produced benign tumors.

Agents either chemically similar to known carcinogens or having molecular constituents which when free possess carcinogenic properties or can be converted by metabolic processes into carcinogens also should be investigated with special care.

The following general experimental conditions should be observed in testing food additives and contaminants for carcinogenic properties:

1. Use of several species (mouse, rat, hamster, guinea pig, rabbit, dog, cat, monkey, chicken, etc.), the selection being made, if possible, on the basis of metabolic similarity to man concerning the chemical to be tested. Animals of both sexes should be employed.

2. Several strains of the same species may be employed, having different susceptibilities to development of "spontaneous" tumors at different sites. The incidence rate and age and sex distribution of such tumors under standardized experimental conditions must be known so that any deviations from normal conditions of occurrence under the influence of the test chemical may be assessed.

3. Pregnant animals should be included in the experiment so as to ascertain any neoplastic responses in the offspring from the chemical administered to the mother. The offspring should be kept under observation for its entire lifetime.

4. Various routes of administration of the chemical should be used. The alimentary route always should be included.

5. The vehicle used depends, in part, upon the route of administration. Depending upon the route of administration, it may be advisable to choose a vehicle which retards removal from the site of administration (subcutaneous), so as to favor the development of tumors at the site of injection, or it may be selected for increasing and accelerating the absorption of the chemical (oral route).

6. All animals having an average life span of one to five years should be allowed to live out their full life span, while continuously exposed to the agent tested. Animals with long life spans, such as dogs, should be observed for a minimal period of 10 years.

7. Competent postmortem examination should be performed on all animals, and histologic studies should be made on an adequate number of animals regardless of whether they show or do not show gross pathologic changes. However, all animals with any suspicious lesion should always have a histologic examination of all organs.

8. Whenever the metabolism of the chemical to be studied is not known, such investigations are necessary (urine, feces, blood, depot organs) because of the species-specific properties of carcinogens. False-negative results may thereby be avoided or properly assessed.

9. The number of animals used in individual experiments should be sufficiently large to ensure the survival of an adequate number of animals, if necessary, to an advanced adult age.

10. Since experimental animals, like man, develop "spontaneous" tumors, i. e., tumors of unknown etiology, in all experiments an adequate number of animals of both sexes and ages should be used as controls. There also should be vehicle controls if the test agent is administered in a vehicle other than distilled water or isotonic saline solution. Since many exogenous carcinogens are presumably rather weak ones, it is advisable that the number of control animals be at least equal to that of the test animals, because the yield of tumors in such experiments will be relatively low and the age of the animals at their appearance comparatively advanced; i. e., there will in all probability be only a small number of animals surviving to an advanced age. The same precaution is indicated if animal strains are used for which insufficient data on the incidence rates, sites, age, and sex distribution of "spontaneous" tumors are not available.

11. In the assessment of the relative degree of the potential carcinogenic hazard which might be connected with the use of a prospective food additive, proper cognizance should be taken of the fact that the total daily and lifelong exposure to any particular chemical often occurs from different products and various routes (cutaneous, respiratory, alimentary). Exposure to such prospective food additives may be complicated by contact with other food ingredients which differ chemically from the additive under test but show, nevertheless, a carcinogenic

action, similar to or identical with it. Neglect of the possible existence of such synergistic effects may lead to serious errors of judgment.

5. Relative Significance of Food Additives and Contaminants as Possible Cancer Hazards

From a public health viewpoint, the relative significance of a chemical as a potential carcinogen depends to a definite degree upon the number of persons who may become exposed to such a substance through some channel, as well as upon the technical and economical ability to control any cancer hazard connected with its production, use, and consumption. In a graduated scale of the relative significance of potential environmental carcinogenic agents from a public health viewpoint, the highest priority for carcinogenic screening should be extended to those agents with which large parts of the general population have frequent and prolonged contact, whose possible carcinogenic effects on man can least readily be ascertained, and which for this reason are most difficult to control by preventive methods. Chemicals included in this group are those which enter the general human environment or every home in the form of consumer goods or as environmental contaminants. Agents of this type are the large group of chemical additives and contaminants of foodstuffs, in addition to many environmental poisons, water, air and soil pollutants, household drugs, sanitary supplies, cleaning agents, polishes, paints, and cosmetics.

If one adopts the principle that the protection of the health of the general public deserves foremost attention, the following considerations may profitably be used as guides in arriving at intelligent and rational decisions when assessing the significance of these potential or actual hazards.

1. Carcinogens vary greatly in their relative potency. Coal tars of different derivations and obtained under different production methods, for instance, vary greatly in their relative carcinogenic potency in man and experimental animals. Coal tars obtained in high-temperature gas-house retorts or from coking operations have in general a considerably higher carcinogenic potency than coal tars manufactured in low-temperature retorts. Coal tars are, in turn, usually more potent than wood tars or vegetable tars or tars obtained in the fractionation of petroleum.

Some carcinogens are so potent that frequent exposures to minute amounts may produce cancers. Experiments of Shear have shown that as little as 0.004 mg. of 1,2,5,6-dibenzanthracene incorporated in a cholesterol pellet and placed into the subcutaneous tissue of mice will elicit cancer formation at the site of deposition, while 1 mg. of methylcholanthrene in tricaprylin subcutaneously injected gives a 100% tumor yield in animals thus treated. Observations made among workers exposed to β-naphthylamine suggest that a daily exposure to 0.3 to 0.6 mg. of this chemical for a period of one month only may represent an effective carcinogenic contact and may result after a latent period of one to several decades in the development of bladder cancers in some persons.

2. Dose observations made in experimental animals are not directly applicable to man, since there exist marked differences in potency of a particular chemical for various species. While the skin of man and mice seems to be highly susceptible to the carcinogenic action of coal tars, the carcinogenic effectiveness of these agents is definitely weaker in rabbits and practically absent in monkeys and rats. Butter yellow, which readily produces, under proper dietary conditions, benign and malignant hepatomas in rats, is less effective in this respect in mice and ineffective in rabbits. Methylcholanthrene, which promptly elicits skin cancers

in mice after cutaneous applications of this chemical, does not produce such neoplastic responses when applied to the skin of rats, although this species reacts readily with cancer formation when methylcholanthrene is subcutaneously injected. Prolonged exposure to ultraviolet radiation has a carcinogenic effect upon the skin of man, mice, and rats, but so far has failed to cause similar cancerous reactions in the skin of rabbits and guinea pigs. β-naphthylamine causes cancer formation in the bladder of man and dogs, and perhaps also in rabbits, but is ineffective when given orally to rats and mice. The reason for this divergent species-specific behavior toward the carcinogenic action of β-naphthylamine is apparently related to species-specific differences in the metabolites formed from this chemical. Those species which are susceptible to the carcinogenic action of β-naphthylamine metabolize a part of this chemical to the carcinogen, 2-amino-1-naphthol sulfate, which is excreted in the urine, while the refractory species fail to do so, or produce only minute amounts.

3. Repeated exposures to carcinogens produce a cumulative carcinogenic effect in the exposed tissues. Observations made on man and experimental animals indicate that biologic effects of ionizing radiation, including carcinogenic effects, often depend upon a cumulative action of repeated exposures to these agents sustained at regular or irregular intervals, which may be as long as several years. Cells once exposed to a carcinogen seem to retain the entire or a considerable portion of the initial effect exerted by individual exposures, even if these by themselves may be insufficient to elicit a neoplastic response. The latent state of specific carcinogenic sensitization of cells previously subjected to carcinogenically subminimal doses has been demonstrated by the carcinogenesis-promoting effect exerted upon such sensitized cell complexes by specific chemical agents, such as croton oil, iodoacetic acid, chloroacetophenone, sorbitan monolaurate, polyoxyethylene sorbitan monostearate, and certain distillation residues of catalytically cracked petroleum, which by themselves are not carcinogenic and which are known as co-carcinogens, accelerators, or promoters (Shubik). Subeffectively exposed cells can be challenged into carcinogenic activity either by additional subminimal carcinogenic exposures or by contact with specific promoting chemicals and thus the latent biologic effect of the initial sensitization be demonstrated.

4. Actual exposure to a dietary carcinogen does not always stop with the cessation of environmental contact, since some of these chemicals are not metabolically destroyed or excreted but are retained in active form in certain tissues, from which they may gradually be mobilized long after the environmental exposure has ceased. It is well known that arsenicals, for instance, may be retained in certain tissues over periods of years, that benzene may be isolated from bone marrow up to several months after cessation of exposure, that radioactive substances, once deposited in bony tissue, are immobilized there for periods of years, that certain carcinogenic chlorinated hydrocarbons, including those used as pesticides, may be accumulated and stored for many months in the fat tissue, and that certain carcinogenic azo dyes combine with proteinic elements of liver cells.

5. Exposure to a dietary carcinogen may be complicated by occupational, medicinal, cosmetic, sanitary, or environmental contact with the same chemical or some other synergistically acting chemical.

Exposure to arsenicals present as pesticide residues in foodstuffs may be complicated by simultaneous or heterochronic contacts with arsenicals contained in medicinal and cosmetic agents, in insecticides applied for occupational or hobby

reasons, or in products encountered during numerous occupational activities. Hepatotoxic effects related to chlorinated hydrocarbons present in foodstuffs as pesticide residues may be accentuated and become manifest by contacts with similar chemicals handled either for occupational reasons in various industries or in the household as dry-cleaning agents, insecticides, solvents of floor waxes and paint removers, or by various chemically dissimilar hepatotoxic substances ingested for medicinal or dietary reasons.

It is neither scientifically sound nor practically wise on the basis of the recorded facts and observations to establish maximal permissible amounts of carcinogenic chemicals in foodstuffs by using a rigid formula which fixes such amounts as a fraction of the minimal carcinogenically effective dose found for any particular chemical in experiments with one, or even several, species. The adoption of such a practice may indeed lead at times to serious cancer hazards among some undetermined and undeterminable portion of the general population consuming carcinogenically contaminated foodstuffs.

6. It is perhaps possible to enforce, to a reasonable degree, laws concerning the maximal content and adequate purity of food additives and contaminants in foodstuffs merchandized by relatively large trade organizations dealing in large quantities in nation-wide and interstate commerce and using standardized and well-controlled methods of processing, handling, and shipping. Considerable difficulties in this respect may be encountered, on the other hand, regarding the proper supervision of foodstuffs produced and sold on a local level. The mere passage of laws establishing standards in such matters without providing adequate means to enforce them might produce in the population a deceptive impression of safety, and would represent an unrealistic approach to a public health problem of great human and economic importance. The most effective method of control of health hazards of this type doubtless is found under such circumstances in a complete elimination of the dangerous agents from the human environment, wherever such a procedure is possible.

7. The inherent soundness and fairness of this proposition is supported by the following considerations: Since many foodstuffs containing artificial food additives and contaminants are not adequately labeled as to the amount and type of chemicals added to the natural food products, the general consumer is relatively rarely able to make any intelligent selection between different products of the same type, particularly between "natural" foodstuffs and "artificially modified and contaminated" ones. Indeed, in many instances, he may have little choice in such matters, because all, or nearly all, foodstuffs of certain types which he is able to purchase are of the contaminated, or modified, variety. The consumer under such circumstances is a member of a "captive" population which may be subjected to potential, long-delayed health hazards that he neither has consented to nor is able to avoid. He is, moreover, incompetent to judge the character and manifestations of any health hazards which may be associated with the consumption of such agents. It is also for these reasons that the general public can expect that all chemical additives and contaminants are subjected to comprehensive and thorough studies for toxic, carcinogenic, and co-carcinogenic properties before they are used or introduced in human foodstuffs.

There can be little doubt that in the past this type of protection has not been adequate, since recent years have brought a considerable number of changes, including withdrawals and prohibitions of previously permitted additives, in the

regulations pertaining to food additives and contaminants. The considerable differences in the type of food laws enacted in various countries concerning health and cancer hazards from food additives and contaminants attest to the prevailing uncertainties in such matters and the apparent lack of adequate and valid factual evidence upon which to base rational and effective regulations. Although it cannot be expected that even under the best and most efficiently working regulations concerning health and cancer hazards from food additives and contaminants complete protection under all circumstances for the general population can be attained, there can be no doubt that the present unsatisfactory situation in carcinogenic respects can greatly be improved.

In formulating a program for studying the numerous food additives and contaminants for carcinogenic properties, proper cognizance should be taken of the fact that the absence of pertinent observations concerning the occurrence of cancers in connection with any particular chemical among the general population, although perhaps used for many years, does not entirely exclude the possible existence of cancer hazards. This conclusion is indicated from observations made in connection with occupational cancer hazards in various industries, in which sometimes the discovery of occupational cancers was missed for many years, until thorough and competent epidemiologic surveys of the population at risk were made. It is possible, moreover, since the cause of the majority of human cancers is still unknown, that environmental factors, including those of dietary nature, may play a significant direct or indirect etiologic role.

It is unlikely, however, from an application of our present knowledge of environmental carcinogens, that many of the presently used additives and contaminants of foodstuffs, especially most of those of purely inorganic nature, unless they are radioactive or belong to the group of carcinogenic metals (nickel, chromium, selenium, beryllium, cobalt, arsenic), introduce any carcinogenic hazard into the general food supply and, therefore, deserve any immediate attention. The large number of these agents, as well as the complexity and costliness of the biologic testing for carcinogenic properties of any single agent, moreover, precludes for merely practical reasons, at the present time, any large-scale attack of the problem on the entire front. It is quite obvious that under the existing conditions a step-by-step procedure will have to be adopted and that investigative efforts would best be expended for the time being on those circumscribed groups of chemicals which from the already available information have furnished carcinogenic or co-carcinogenic agents, i. e., synthetic dyes, chlorinated pesticides, animal and plant hormones, and detergents.

6. Investigative Scheme for Studying Cancer Hazards from Food Additives and Contaminants

An investigation of food additives and contaminants as cancer hazards to the producer of the chemicals and of the foodstuffs, their processors, handlers, and consumers, has to include and evaluate data of four main aspects:

1. Chemical data pertaining to the chemical composition and structure of the substance; its reactivity with other chemicals which may be encountered in foodstuffs under the conditions prevailing during the processing of foodstuffs and in the preparation of food for human consumption; its production methods; its degree of purity and uniformity (isomers); the amounts and types of impurities; the type and sensitivity of chemical methods available for its identification; its

stability when exposed to light and heat, and the chemical nature of its conversion products and potential metabolites.

2. Data on the acute and chronic toxicity of the substance in man and experimental animals under various conditions of exposure, its metabolism, its effects upon organs and tissues, its allergenic properties, its routes and products of excretion, and its sites of deposition.

3. Epidemiologic and symptomatic data on cancer occurring among human populations exposed to the chemical during its production; its employment in various consuming industries, in agriculture, in food and processing plants, and in merchandizing establishments, and by the general public using industrial goods containing the chemical and consuming foodstuffs into which it has intentionally or unintentionally been introduced.

4. Evidence on cancer in experimental animals which have been exposed to it under varying conditions and amounts and for an adequate period of time.

Sufficient data on these various aspects must be available before definite decisions can be made concerning the suitability of a chemical as a food additive or as a pesticide or some other environmental poison, and as to the type of regulations under which it may be used (maximal permissible dose, type of foodstuff, etc.).

The investigative program outlined is obviously of a scope and character which make its routine application to a large number of chemicals impractical and uneconomical. It becomes necessary, therefore, for the ensuring of an adequate health protection of the general public, to limit the number of permitted food additives and contaminants to the smallest number which still meets the essential economic requirements of the industries producing, processing, and merchandizing foodstuffs and the protective demands of the general public consuming them. The following program for cancerigenic studies on synthetic food dyes may serve as an illustration of the investigative pattern and scientific and practical considerations which have to be applied to this end.

A Model Cancer Research Program into Synthetic Food Dyes

1. *Chemical and Technological Aspects.*—As a prerequisite of a rational approach to the study of potential cancer hazards from synthetic food dyes, a serious attempt should be made to reduce the number of dyes used for the coloring of foodstuffs, drugs, and cosmetics, i. e., dyes which enter the human body, to the absolute minimum. If at all possible, general agreement should be reached on an international level concerning the particular dyes to be placed on the international list of permitted dyes. Since the use of different raw products and manufacturing methods may result in finished dyes of varying composition, i. e., relative amounts of isomers, homologues, and impurities, often greatly differing in their biologic reactivity, organ specificity, and metabolites, it may be that international uniformity will be desirable also in these respects. Chemical and bioassay methods of testing for purity, types, and amounts of impurities, and for toxicity should be standardized, so that every food dye used is everywhere chemically and biologically of identical quality. Such adjustments in our at present rather complex, if not confused, food economy of dyes would distinctly enhance the conclusive and general value of investigations conducted on population groups, as well as experimental animals, concerning potential cancer hazards associated with these food additives.

The degree of exposure, and thereby the extent of the potential cancer hazard

from synthetic food dyes, depend upon the chemical types, total quantities used, and maximal amounts of individual dyes permitted in various foodstuffs, in addition to the types, numbers, and quantities of foodstuffs to which dyes may be added. The following data may illustrate this point in regard to any attempt aimed at drawing general conclusions from information obtained from different countries.

TABLE 1.—*List of Food Dyes Certified During 1955 by the Food & Drug Administration, U. S. Department of Health, Education, and Welfare*
Oct. 1-Dec. 30
Jan. 1-Dec. 30

FD&C Name	Pounds	Pounds (Common Name)
FD&C Blue No. 1	6,006	29,681 (Brilliant blue FCF)
FD&C Blue No. 2	3,343	4,310 (Indigotine; indigo carmine)
FD&C Green No. 1	1,120	2,239 (Guinea green B)
FD&C Green No. 2	----	1,189 (Light green SF yellowish)
FD&C Green No. 3	436	1,599 (Fast green FCF)
FD&C Orange No. 1	81,186	187,584 (Orange I)
FD&C Orange No. 2	7,647	8,163 (Orange SS)
FD&C Red No. 1	24,254	113,063 (Ponceau 3R)
FD&C Red No. 2	122,809	431,547 (Amaranth)
FD&C Red No. 3	5,041	20,094 (Erythrosine)
FD&C Red No. 4	8,625	50,630 (Ponceau Sx)
FD&C Red No. 32	10,000	56,495 (Oil red XO)
FD&C Violet No. 1	-------	3,196 (Anthraquinone violet B)
FD&C Yellow No. 1	-------	1,473 (Naphthol yellow S)
FD&C Yellow No. 3	20,275	49,931 (Yellow AB)
FD&C Yellow No. 4	22,379	55,562 (Yellow OB)
FD&C Yellow No. 5	67,837	301,091 (Tartrazine)
FD&C Yellow No. 6	57,860	228,751 (Sunset yellow FCF)
FD&C—Lakes	-------	2,447

Five (orange I; ponceau 3R, amaranth, tartrazine, sunset yellow FCF) of the 18 food dyes certified during 1955 furnished about 1,260,000 lb. out of a total of approximately 1,547,000 lb. Similar quantitative discrepancies become evident from an analysis of the Australian figures on food dyes imported during 1950 to 1954, although the much larger number of food dyes formerly used in this country makes for a wider spread in the quantities of the individual dyes used. Five dyes (naphthalene scarlet RS, tartrazine, naphthalene orange G, nigrosine, and carmoisine) accounted for almost half the total amount of food dyes imported annually during a five-year period (1950-1954). Since the annual consumption of all types of food dyes per head of population stands at 4.5 gm. in the United States, is about 4 gm. in Australia, and is approximately 2.8 gm. in Israel, national differences in the quantities and qualities of dyes incorporated into foodstuffs in different countries may be of distinct importance in influencing relative potential cancer hazards from such sources on a regional basis.

This situation is further complicated by the fact that marked variations exist in the types and numbers of food products which may be colored by the various dyes in different countries, and that regional differences in food habits contribute an additional factor modifying possibly the degree of potential exposure to carcinogenic dyes. The list of dyes and the food products which are being colored with them according to information received from 29 countries, presented in Table 2, may help to illustrate this point.

2. *Toxicological Aspects.*—While experiments designed for determining the chronic toxicity of a chemical only exceptionally yield information also on carcinogenic potency, they, nevertheless, may furnish important leads in this respect and thus may be used as a rough screening procedure for carcinogenic properties. Thus, dyes which display a hepatotoxic action should be studied thoroughly under

TABLE 2.—*Selected List of Food Dyes and of Food Products Dyed with Them*

Color	Color Index	Commercial Name of Dye	Chemical Name of Dye	Food Products	No. of Countries out of 29 Surveyed
Yellow	655	Auramine O	Tetramethyl-diamino phenonimide hydrochloride	Cordials	6
	10	Naphthol yellow S (Manchester yellow, Martius yellow, acid yellow S, citronin A, sulfur yellow)	Potassium or sodium salt of 2,4-dinitro-1-naphthol-7-sulfonic acid	Confectionery, pastas, macaroni, spaghetti, baked goods, beverages	23
	640	Tartrazine (tartar yellow, tartratrol yellow, tartrazine O, hydrazin yellow C)	Trisodium salt of 3-carboxy-5-hydroxy-1-*p*-sulfophenyl-4-*p*-sulfophenylazo-pyrazolone	Custards, pickles, cordials, cordial extracts and crystals, jellies, junket tablets, ice cream and ice cream toppings, milk-bar syrup, pastry cook fillings and spread, alcoholic and non-alcoholic beverages, solutions for house use	23
	----	Sunset yellow FCF (Para orange, sun yellow, yellow orange S)	1-Aminobenzene-4-sulfonic acid-azo-2-naphthol-6-sulfonic acid sodium	Milk-bar syrups, blanc mange powder, jellies, frozen desserts, beverages, candy, bakery goods, puddings, cordial extracts, custards, ice cream	16
	16	Fast yellow (acid yellow R), solid yellow, egg yellow, genuine yellow)	Sodium salt of aminoazobenzene disulfonic acid	Like sunset yellow	10
	148	Chrysoine S (resorcin yellow)	*p*-sulfobenzene-azo-resorcinol sodium	Alcoholic beverages	6
	22	Yellow AB	1-Phenylazo-2-naphthylamine	Edible fats, margarine, butter, cakes, biscuits, candy	7
	61	Yellow OB	1-o-tolylazo-2-naphthylamine	Like Yellow AB	7
Orange	150	Orange I (tropaeolin 000, orange S, orange R extra, 1-naphthol orange, Edicol orange 18)	4-*p* (4-hydroxy)-1-naphthylazobenzene sulfonic acid	Fish pastes, carbonated beverages, jellies, confectionery, custards, blanc mange powder, biscuits, cakes, ice cream, cordials, cordial extracts and crystals, ice cream toppings, milk bar syrups, sausage casings, puddings, frozen desserts, solutions for home use, soft drinks	15
	----	Orange SS	1-o-tolylazo-2-naphthol	Cheese, margarine, candies, edible fats, oils, external coloring of oranges	6
	27	Orange G (Sudan I)	1-Phenylazo-2-naphthol-6,8-disulfonic acid	Edible oils, fats, external coloring of oranges	7
Red	184	Amaranth (Bordeaux S, naphthol red S, Victoria rubin O, azo acid rubin 2B, acid crimson, wool red extra, Edicol amaranth AS	1-Naphthylamine-4-sulfonic acid-azo-2-naphthol-3,6-disulfonic acid (sodium salt)	Black currant jam, candies, cordials, cordial extracts & crystals, jellies, junket tablets, ice cream, milk bar syrups, pastry cook fillings & spreads, ice cream toppings and decorations, maraschino cherries, beverages, sausage casings, bakery goods, solutions for home use	25

TABLE 2.—*Selected List of Food Dyes and of Food Products Dyed with Them (Continued)*

Color	Color Index	Commercial Name of Dye	Chemical Name of Dye	Food Products	No. of Countries out of 29 Surveyed
Red	773	Erythrosine J	Tetraiodofluorescein (sodium or potassium salts)	Fruit glazes, candies, maraschino cherries, nonalcoholic beverages, caramels, fillings of bakery goods, frozen desserts, puddings	25
	79	Ponceau 2R (Ponceau MX, scarlet 2R, cumidine red, Edicol ponceau AS)	Xylidine-azo-2-naphthol-3,6-disulfonic acid (sodium salt)	Fish pastes, cordials, syrups, sauces, tomato products, jellies, candies, jams, maraschino cherries, ice cream, ice cream toppings, milk bar syrups, extracts, essences, marmalades, solutions for home use	7
	80	Ponceau 3R	1-Pseudocumylazo-2-naphthol-3,6-disulfonic acid (sodium salt)	Candies, jellies, canned fruits, cordials, jellies, maraschino cherries, beverages, bakery goods, puddings, sausage casings	19
	*	Ponceau 8X	2-(5-sulfo-2,4-xylylazo)-1-naphthol-4-sulfonic acid (disodium salt)	Candies, maraschino cherries, frozen desserts, icings, fillings and decorations of bakery goods, puddings	15
	179	Carmoisine (azo rubin, fast red S, crimson cardinal 3B)	1-Naphthylamine-4-sulfonic acid-azo-1-naphthol-4-sulfonic acid (sodium salt)	Cordials, cordial extracts and crystals, jellies, blancmange powder, custard powder, junket tablets, ice cream, ice cream toppings, milk bar syrups, pastry cook fillings and spreads, soft drinks, candies, solutions for home use	12
	749	Rhodamine B	Tetraethyldiamino-o-carboxyphenyl-xanthylium chloride	Fish pastes, canned meats, ice cream, ice cream cakes, canned pastes, meat products, candies, biscuits, cakes, solutions for home use	5
	73	Oil Red XO (Sudan II)	1-xylylazo-2-naphthol	Cheese, edible fats, oils, candy, bakery goods, external color of oranges	6
	57	Lissamine Red 6B (amidonaphthol red 6B, azofuchsin 6B extra, etc.)	Acetyl-*p*-phenylene-diamino-azo-acetyl-1-amino-8-naphthol-3,6-disulfonic acid (sodium salt)	Ice cream cake decorations, drinks, jellies	3
	182	Naphthol red (fast red E, solid red E, naphthion red, etc.)	4-Sulfo-2-naphthalene-azo-2-naphthol-6-sulfonic acid (sodium salt)	Marmalades, jellies, essences	9
	185	New coccin (ponceau 4R, cochineal red A, crocein scarlet 4BX, Victoria scarlet)	1-(4-sulfo-1-naphthylazo)-2-naphthol-6,8-disulfonic acid (disodium salt)	Marmalades, essences, jellies, sausage casings	16
Blue	1180	Indogotine (indigo carmine)	5,5-indigotin disulfonic acid (sodium salt)	Black currant jams, jellies, candies, ice cream, ice cream cake decorations, biscuits, soft drinks, puddings, bakery goods	24

TABLE 2.—*Selected List of Food Dyes and of Food Products Dyed with Them (Continued)*

Color	Color Index	Commercial Name of Dye	Chemical Name of Dye	Food Products	No. of Countries out of 29 Surveyed
Blue	671	Brilliant Blue FCF (patent blue AE, azure blue, etc.)	4-(4-(N-ethyl-*p*-sulfobenzylamino)-phenyl)-(2-sulfonium-phenyl)-methylene)-(1-(N-ethyl-N-*p*-sulfobenzyl)-2,5-cyclohexadienimine (disodium salt)	Icings, cordials, cordial extracts and crystals, jellies, ice cream, ice cream toppings, milk bar syrups, candies, cake decorations, frozen desserts, soft drinks, puddings, bakery goods, solutions for home use	15
	672	Patent blue V (azure blue VX)	4-(4-(N-diethylamino)-phenyl)-1-(4-sulfo-2-sulfonium-phenyl)-methylene)-N-diethyl-2,5-cyclohexadienimine (monosodium salt)	Chocolate, candies	8
Violet	698	Acid violet 5BN	4-(4-(N-ethyl-*p*-sulfobenzylamino)-phenyl)-(4-(N-ethyl-*p*-sulfonium benzylamino)-phenyl)-methylene)-N,N-dimethyl-2,5-cyclohexadienimine (monosodium salt)	Chocolate, candies, jellies, frozen desserts, beverages, bakery goods, icings, puddings	9
Green	666	Acid green J (Guinea green B)	4-(4-(N-ethyl-*p*-sulfobenzylamino)-diphenylmethylene)-(1-(N-ethyl-N-*p*-sulfonium-benzyl)-2,5-cyclohexadienimine (monosodium salt)	Cordials, jellies, soft drinks, candy, bakery goods, frozen desserts	18
	----	Patent green FCF (fast green FCF)	4-(4-N-ethyl-*p*-sulfobenzylamino)-phenyl)-(4-hydroxy-2-sulfonium-phenyl)-methylene)-(1-(N-ethyl-N-*p*-sulfobenzyl)-2,5-cyclohexadienimine (disodium salt)	Candies, jellies, desserts, bakery goods	10
	737	Lissamine green BS (wool green BS, etc.)	Tetramethyl-*p*-aminodiphenooxydisulfo-naphthofuchsonium (sodium salt)	Canned peas and other vegetables and fruits, ice cream toppings	5
	670	Light green SF (fast green N, acid green GG, etc.)	4-(4-(N-ethyl-*p*-sulfobenzylamino)-phenyl)-(4-sulfonium-phenyl)-methylene)-(1-(N-ethyl-N-*p*-sulfobenzyl)-2,5-cyclohexadienimine (disodium salt)	Candies, essences, cordials, biscuits, cake, jellies, maraschino cherries, frozen desserts	12
	657	Malachite green (Diamant green, solid green B, etc.)	*p,p'*-Tetramethyldiaminotriphenylcarbinol anhydride	Alcoholic and nonalcoholic beverages	6
Brown	332	Bismarck brown	*m*-Phenylenediamine-disazo-bi-*m*-phenylenediamine chlorohydrate	Custard powder, chocolate, candies, casings of sausages, jellies, puddings, milk bar syrups, blancmange powder, fish products	4
Black	865	Nigrosine	Complex azine dye	Licorice, candies, jellies, aniseed balls, ice cream, pastry cook fillings and spreads, syrups, jams	3

various nutritional conditions and in several species for possible carcinogenic action on different tissues and organs, especially the liver, bladder, bone marrow, and intestine. Similar considerations should be applied if a dye exhibits estrogenic, allergenic, or mitotoxic qualities. Druckrey and Schmähl recently pointed out in this connection that 4-amino compounds of stilbene, azobenzene, triphenylmethane, or diphenylmethane elicit cancers in various species, while the corresponding 4,4′-dioxy compounds of these basic molecules, such as 4,4′-dioxy-triphenylmethane, are estrogenic. There exists a striking morphologic similarity between the highly cellular bone marrow with maturation arrest seen in allergic agranulocytoses caused by aromatic drugs and the changes observed in the preleukemic state of persons exposed to ionizing radiation and to chronic benzolism. The ambivalent properties of many carcinostatic agents suggest that chemicals which appear to be mitotic poisons may under proper conditions of exposure prove to have carcinogenic qualities.

The occurrence of congenital anomalies, such as hydrocephalus, spina bifida, clubfoot, anophthalmia, cardiovascular and genitourinary defects, and displaced hindlimbs, in the offspring of rats injected during pregnancy with aqueous solutions of trypan blue or azo blue, i.e., dyes which have in common a 3,3′-dimethyl-4,4′-diazobiphenyl group, also deserves attention, since parenterally administered trypan blue elicits in rats reticulum-cell sarcomas (Wilson; Gillman, Gillman, and Gilbert; Simpson). The teratogenic effect of these dyes on the fetus, moreover, demonstrates that they penetrate the placental barrier.

While such colloidal dyes when fed usually do not pass through the intestinal mucosa, they are absorbed and produce widespread vital staining of tissues when they are administered along with detergents. Since surfactants are food additives and contaminants, this observation may be of significance in relation to human exposures to food dyes which either are present in food in a colloidal state or under ordinary conditions are not readily solubilized in the intestinal tract, and thus upon ordinary oral introduction may prove to be noncarcinogenic, although they display this property when administered by subcutaneous injection (light green SF, brilliant blue FSF, fast green FCF). Detergents in foods may act under such conditions as co-carcinogens or carcinogenic promoters, as shown in experiments on mice given Atlas Tweens and Spans together with a carcinogenic polycyclic hydrocarbon (Setälä). Nutritional carcinogens, moreover, may be converted thereby from local alimentary agents into general or systemic agents. It seems to be desirable, therefore, that chronic toxicity tests include feeding experiments of dyes with surfactants used as food additives or present in foodstuffs as contaminants so as to ascertain whether the toxicity of dyes is potentiated through the simultaneous presence of detergents in the food and whether dyes ordinarily not absorbed enter the human organism under such circumstances and can be demonstrated in the blood or in the urine.

Since the toxic properties of a chemical are not necessarily related to its possible carcinogenic properties, it is fundamentally unsound from a scientific viewpoint, and dangerous from a practical one, to calculate safety levels of carcinogenic dyes or any other food additive and contaminant in terms of fractions of their minimal toxic doses. This conclusion deserves special emphasis in view of the fact that 2-naphthylamine, one of the various constituents of the molecules of several food dyes, possesses a remarkably low toxicity, while being, on the other hand, one of the most carcinogenic substances known.

3. *Screening Tests of Dyes for Carcinogenic Properties.*—In the designing of screening tests of dyes for carcinogenic properties, three main facets of this problem must be explored: chemical aspects of the dye, biochemical aspects, and biologic aspects. Information obtained from these three approaches must be supplemented, if possible, by the results of epidemiologic surveys conducted on human population groups exposed for some reason to the same or related chemicals.

A. Chemical Aspects: The first step in the chemical approach is the establishment of the general type to which any particular dye may belong.—i.e., whether it is a mono-,di-,tri-,or polyazo compound; a triphenylmethane, diphenylmethane, xanthene, pyrazolone, or anthraquinone derivative, and whether it is a chemical entity or a mixture of isomers. Carcinogenic agents have been found among the members of the first three groups listed. An analysis of the composition of the dye then should be made, to ascertain whether it contains as a component part of its molecule, 2-naphthylamine, 1-naphthylamine, 2-amino-1-naphthol, 1-amino-2-naphthol, or two benzene rings connected by an azo group, a stilbene group, or a single bond. Compounds with such constituents and configurations may be suspected of possessing carcinogenic properties. The presence of a nitro group in the side-chain of a ring in place of an amino group does not guarantee the noncarcinogenicity of a dye, because it has been shown that nitro- or isocyanate groups attached to aromatic rings are reduced in the body to amino groups. It is also not safe to assume that sulfonation of a dye destroys invariably its carcinogenic properties, because several sulfonated dyes (light green SF, Guinea green B, trypan blue) have been shown to be carcinogenic to experimental animals. Since most food dyes contain a considerable amount of impurities, attempts to ascertain their chemical character should be made, since they may account for, or contribute to, any carcinogenic quality a dye may have.

It is, moreover, essential to know whether a particular dye is fat- or water-soluble, whether aqueous solutions are true or colloidal solutions, and whether the dye is used in dissolved or pigmentary form. These factors exert an influence upon the absorption of a dye in the gastrointestinal tract. Ingested matter is subject to acid and alkaline environments in the alimentary tract; hence in vitro tests are indicated to ascertain the solubility and stability of a dye at various pH levels, especially when exposed to hydrochloric acid. Tests may also be made to show whether or not an azo dye, such as yellow AB, may form in an acid medium, when in contact with aldehydes derived from carbohydrates, amidoazo complexes, which usually have great chemical stability. It is necessary, moreover, to test the stability of food dyes after they have been exposed to rather high temperatures, such as those encountered during frying, roasting, and baking in the presence of oxygen and carbon monoxide. Whenever a food dye is customarily used as a part of a mixture of several dyes, corresponding experiments should be performed on such dye combinations, since the possible reaction products of the various dyes may interact with each other and form new compounds not formed when only one dye is present. Finally, it may be desirable to conduct exploratory investigations concerning the affinity of a dye for proteins, such as albumins and globulins, the formation of dye-protein complexes, and the chemical groups through which such bindings are accomplished.

B. Biochemical Aspects: In the biochemical study of dyes, the determination of their metabolites in man, experimental animal species, and under various conditions of metabolic strain, such as types of malnutrition, impaired liver and

kidney function, and endocrine disturbances, represents one of the major approaches for assessing their carcinogenic potentialities. The demonstration of a known carcinogenic metabolite, such as 2-amino-1-naphthol, in the urine following the ingestion of a dye containing in its molecule a recognized human carcinogenic agent, such as 2-aminonaphthalene, would provide adequate proof of the existence of a cancer hazard to man from such an alimentary source. It should be kept in mind, however, that special dietary conditions or metabolic disturbances may alter the chemical type of metabolite formed and may make a substance which is noncarcinogenic under normal conditions into one which is carcinogenic and vice versa. The metabolism of the azo carcinogen *o*-aminoazotoluene is altered in rats by dietary conditions. With a low-protein diet the destruction of the dye is decreased and the amount of protein-bound dye in the liver is increased. High riboflavin-protein supplements, on the other hand, decrease the amount of *m*-toluidine and increase the amount of the carboxy derivative excreted, while, at the same time, the animal becomes increasingly refractory to the hepatocarcinogenic action of the dye.

Metabolic studies of 2-aminonaphthalene on various species in relation to its species-specific carcinogenic potency have revealed that in man and dog 2-aminonaphthalene is metabolized and excreted in the urine mainly as 2-amino-1-naphthol sulfate, which has been shown to be a carcinogen when introduced directly into the bladder of rats (Bonser) and which, at least in part, accounts for the development of bladder cancer in man and dogs exposed to this aromatic amine. Other species (monkeys, rabbits, rats), which metabolize 2-aminonaphthalene by forming 2-amino-6-naphthol, are, in contrast, refractory to its carcinogenic action. This observation, moreover, indicates another important aspect of metabolic studies on food dyes, namely, the necessity of selecting for bioassay purposes those species which form metabolites identical with those produced by man. Rats, for instance, are unsuitable for investigations on dyes in which the active carcinogenic principle might be represented by 2-aminonaphthalene (yellow AB and yellow OB). Since many of the food dyes used are nonstandardized mixtures of isomers and impurities, the isolation and identification of all metabolites formed are, as a rule, quite difficult. It is usually not possible to account by such studies for a considerable portion of the dye ingested, even when urine, blood, feces, and tissue analyses are made.

Biochemical studies on the fate of food dyes should include examinations of various tissues and organs for dye retained in them in dissolved or bound form. Fat-soluble dyes, for instance, may be stored in the fat tissue, while certain azo dyes of the butter yellow variety are, in part, bound to the protein of liver cells. Other dyes are known to have special affinities for bony structures or to form complexes with plasma proteins.

Since different dyes have different metabolic pathways, different organ and tissue affinities, and different excretory routes, which include the glandular epithelium of mucous membranes, the use of dyes specially prepared with radioactive components may be advantageous not only for studying the distribution pattern of ingested dyes but, whenever they should be carcinogenic, to ascertain also the metabolic background which may underlie the species-specific aspect of this property, as well as the often-observed shift in carcinogenic target organ and tissue, depending on the species used. While benzidine, for instance, elicits in man and dog primarily cancer of the bladder, it produces hepatic tumors, cancers of the

Eustachian tubes and intestine, and leukemias in rats. Likewise, 4-aminodiphenyl causes the development of bladder cancers in man and dog, whereas, when given to rats, it gives rise to cancers of the intestine and breast (Walpole, Williams, and Roberts).

C. Bioassay Methods for Carcinogenicity: Since food dyes are nutritionally unessential constituents of foodstuffs, it seems reasonable to demand that any dye conveying even a minor degree of health, and especially cancer, hazard to the general consumer be eliminated from the list of permitted food additives. The adoption of such a policy not only would provide the general public with a reasonable amount of protection but at the same time would tend to simplify to some extent the amount and caliber of experimental procedures required for the testing of dyes for carcinogenicity by bioassay methods. Under such conditions the main emphasis of such tests would be placed upon qualitative aspects, and not on quantitative ones.

In these tests, young animals of several species and both sexes should be employed. They should be selected for their similarity in metabolism of a particular dye studied to that prevailing in man. The dye should be administered in one set of experiments in maximal amounts tolerated over long periods of time, i.e., the entire life span. The route of introduction chosen for this purpose may not necessarily be the oral route, but should represent, rather, that route which creates the severest, and most continuous and effective exposure. The production of a parenterally placed depot or depots from which the dye can slowly be taken up into the organism may create in many instances, but not always, such exposure conditions. In one set of experiments the dye should be given by the oral route and should be incorporated into food which is prepared under conditions identical with those observed during the preparation of human meals (heat, shortenings, alcohol, etc.). Female animals should be exposed to the dye before and during repeated pregnancies to study its effects upon the metabolically abnormal organism during pregnancy and to ascertain its effects upon the offspring of such mothers, since, through a transplacental penetration of the dye, cancer may be elicited in the young. The actual existence of such a transplacental transfer of a carcinogen is attested to by the fact that lung tumors developed in the young offspring of female mice which received urethan during pregnancy.

In the evaluation of any cancerous responses which might be observed in different species, consideration should be given to the fact that variations in target organs of different species may be due to species-specific factors, or may be attributable to different routes of introduction used, or may reflect differences in the dose of dye administered or the type of vehicle used. Similarly, differences in the length of latent period in the appearance of tumors in various species and organs may have an endogenous species-specific basis or may be the result of differences in doses given, since large doses seem to hasten the development of cancerous reactions. The marked discrepancies in the relative incidence rates of many cancers in males and females suggest the possibility that at least some part of these differences may be attributable to sex-conditioned differences in metabolism, and that they are not entirely related to variations in the intensity and duration of exposure of the two sexes to specific carcinogenic factors. Attention, therefore, should be paid to any variations in the incidence rate and topographical distribution of tumors appearing in members of both sexes when exposed in approximately the same degree to food dyes.

Since the general human population contains a certain percentage of anatomically, functionally, and metabolically or nutritionally abnormal or defective persons, experiments on animals should include some which are conducted on animals with artifically altered function of metabolically important organs, such as the liver, kidney, gastrointestinal tract, thyroid, adrenal, and pancreas. These experiments are indicated because such abnormal humans may have a reactivity toward carcinogens differing from that of normal ones and may produce metabolites from originally noncarcinogenic or carcinogenic dyes differing in their biologic properties from those normally generated. They may also retain dyes because of impaired function of certain organs over a longer period than normal persons and may excrete dyes through channels which are ordinarily not used. For these reasons their carcinogenic response to food dyes also may fundamentally differ from that of the majority of the population.

It appears from a critical examination of the past work with food dyes and related chemicals (fuchsin; gentian violet; light green SF; butter yellow; azo blue; Bismarck brown; Congo red; Edicol amaranth AS; Edicol orange IS; Edicol ponceau RS; fast brown G; metanil yellow yKS; methyl orange; methyl red; naphthalene fast orange 2GS; naphthol [naphthion] red; naphthol black P; orange II; pigment Bordeau N; ponceau 4GB; scarlet red; Sudan I; Sudan II; Sudan III; yellow AB; yellow OB; auramine; chrysoidine; chrysoidine R; chrysoidine Y; eosin; lithium carmine; naphthol yellow S [Martius yellow] [Hartwell; Hecht]) that only very few, if any, have adequately been studied for carcinogenic properties.

4. *Epidemiologic and Biochemical Studies on Human Populations.*—Unless chemical and biologic studies yield information indicating that a food dye produces metabolites which from other human experience, particularly from the field of occupational cancer, have demonstrated that they are human carcinogens, all experimental data obtained on animals can be given only circumstantial value as to their human significance. It is for this reason most important to obtain, whenever possible, epidemiologic data on the occurrence of cancers by sites in members of population groups especially intensely exposed for some, usually occupational, reasons to the particular dye under test. Unfortunately, it is practically impossible to find a population group whose members have severe contact with one dye only.

Despite this shortcoming, it may be profitable to survey population groups which have contact with restricted groups of dyes identical with or similar to some of the food dyes. Such population groups are found among producers of food dyes, users of food and related dyes in the processing of foodstuffs or in the production and processing of various other industrial substances and products, such as colored flares, smoke signals, fireworks, textiles, paper, plastics, rubber, linoleum, paints, shingles, chinaware, pharmaceuticals, cosmetics, sanitary goods, deodorants, insect repellents, gasoline, cleaning fluids, leather goods, and colored prints. In addition to the prevalence of highly mixed exposures to various dyes, dye intermediates, and rubber antioxidants, some of which have carcinogenic properties, for members of many such occupational groups, other difficulties often present themselves in obtaining or utilizing their records for the purpose indicated. However, whenever such studies may be accomplished, attention should be paid not only to cancerous reactions among members of such groups but also to the incidence and types of allergies and the possible occurrence of congenital malformations and cancers among the children of female members of such groups.

A third population group which may profitably be analyzed for the occurrence of cancers which might be attributable to ingested food dyes are consumers of dyed foodstuffs, particularly if the exposure sustained involves a dye with known carcinogenic properties, such as butter yellow (*p*-dimethylaminoazobenzene), and if the organ or tissue affected by cancer as the result of such contacts is ordinarily one of the rarer sites of cancerous growths, such as the liver. Postmortem observations reported from the Philippines and Austria (Cruz; Zeitlhofer) were interpreted as suggesting that the use of butter yellow in coloring rice dishes is one of the main reasons for the excessive incidence of primary liver cancer in Oriental countries and is responsible for the rise in the incidence of these tumors and of liver cirrhosis found during recent years in Austria, where the dye was used for coloring edible fats. While these claims are provocative, they are at present not supported by adequate factual evidence of any real scientific value. The contentions of dye manufacturers that no special cancer hazards have been observed in past decades among workers employed for many years in azo-dye operations likewise have restricted significance and need confirmation (Wingler).

It may be recalled in this connection that the initial discovery of occupational bladder cancer in dye workers was made in workers engaged in the manufacture of fuchsin (1895) and that, more recently, English investigators noted that workers employed in the production of auramine and magenta (basic fuchsin) also seemed to be affected by cancers of the bladder (Case). However, these dyes belong to the diphenyl or triphenyl group.

In epidemiologic surveys on persons exposed to these dyes, particular attention should be paid to the incidence rates of cancers of the bladder, liver, and intestine and to leukemic and lymphoid neoplastic reactions. Recent observations on man and animals developing synchronic or heterochronic multiple primary cancers following contact with various exogenous carcinogens, including aromatic amines, indicate the necessity of a much broader approach to the problem of potential carcinogenesis following exposure to dyes, involving the phenomenon of multiphasic and hetero-organic carcinogenesis.

Paralleling recently made clinical impressions of a rise in the frequency of multiple primary cancers appearing in persons following a therapeutic arrest of the first-appearing cancer, observations made in the field of occupational carcinogenesis have revealed the fact that workers exposed to various occupational carcinogens seem to have an excessive liability to develop multiple primary cancers. Thus, casuistic evidence recorded the simultaneous occurrence of chronic radiodermatitis, leukemia, and pulmonary cancer in a chemist of a radium ore refinery; the coexistence of chronic arsenic dermatosis and cancers of the skin and lung was noted in workers exposed to arsenicals; the successive appearance of cancers of the skin, stomach, and lung, in a paraffin presser, and the development of cancers in one or two nonurogenous organs (lung, intestine, prostate, etc.) in dye workers following the initial appearance of cancers of the bladder, ureter, or renal pelvis (Mueller; Hueper; Uebelin and Pletscher).

A unitary etiologic causation of such multiple primary cancers is strongly indicated by observations made in experimental animals concerning the occurrence of multiple primary cancers in several organs of the same animal, the development of hetero-organic cancers in different animals, and the shift of target organ in different species when exposed to the same carcinogen. Such findings have been reported particularly often in experiments with various aromatic amines and azo-

compounds (benzidine, 4-amino-diphenyl; 3,2′-dimethyl-4-aminodiphenyl; 2-acetylaminofluorene; 2,3′-dimethylazobenzene) (Spitz, Maguigan, and Dobriner; Walpole, Williams, and Roberts). In some instances of occupational and experimental multiplicity of cancers developing after exposure to one carcinogenic agent, the appearance of the individual tumors seems to adhere to a definite sequence; i. e., bladder cancers in dye workers which represent the first neoplastic manifestation are followed in some cases by the appearance of cancers of various other internal organs, or cancer of the skin precedes the appearance of pulmonary cancer in arsenical workers.

Apart from the definite epidemiologic, etiologic, and medicolegal importance of such observations, the phenomenon of multiple and multiphasic cancerous responses poses an interesting scientific problem. Differences in the time of appearance of the various tumors may be attributable (*a*) to differences in the degree of exposure of various tissues to the same carcinogenic agent, causing discrepancies in the length of their latent period; (*b*) to organ-specific differences in the neoplastic responsiveness to the same dose of carcinogen; (*c*) to the production of several carcinogenic metabolites from the same carcinogen possessing specific affinities to different organs; (*d*) to the presence of traces of carcinogenic or cocarcinogenic contaminants possessing organ affinities differing from those inherent in the carcinogenic chemical; (*e*) to the simultaneous exposure to the same carcinogen by several routes (skin, respiratory, and alimentary tracts); (*f*) to the existence of the carcinogen or several carcinogens, in different physicochemical states (dust, vapor, gas mist, liquid, etc.), resulting in an exposure of several organs or tissues.

These observations and considerations are of importance when studying the potential biologic and carcinogenic significance of different metabolites and excretory products of food dyes contained in and isolated from the urine, feces, or blood of exposed persons.

Conclusions

The facts recorded, the consideration and designs of tests presented, and the large and, in part, enormous amounts of food additives and contaminants used in the production and processing of foodstuffs amply demonstrate the scope and importance of the problem. The program of control outlined, if adopted, would provide a larger amount of protection to the general public, as well as to the producers and users of carcinogenic agents which might be present among existing or future food additives and contaminants. The present state of highly defective knowledge in such matters, as well as the almost universal lack of a comprehensive and competent program of investigation and supervision of health and cancer hazards from food additives and contaminants, present a potentially serious public health problem, especially since the modern food production, processing, and merchandizing methods necessitate the use of many food additives and contaminants for safeguarding an adequate food supply to the urbanized and industrialized population of many countries.

In support of these conclusions and recommendations, it may be pointed out that an ingestive contact with several human environmental carcinogens (arsenicals, aromatic amines, radioactive substances) for occupational, medicinal, and dietary reasons has been shown to be responsible for or to contribute to the development of cancers in various organs and tissues (skin, urinary bladder,

bones). Casuistic data suggest the possible existence of similar relations to cancers of various parts of the alimentary tract for persons exposed to coal tar and petroleum and shale oils (cancer of the lip) and to carcinogenic agents contained in betel nut and khaini quids (cancer of the oral cavity).

Several epidemiologic and statistical data may have a similar significance. Marked differences in the relative frequency of cancer of the stomach have been demonstrated for inhabitants of different countries, which exist even for countries which have a similar racial composition of their populations and medical care and recording systems of comparative quality (Hueper). Since it is scientifically inconceivable to attribute such regional discrepancies to significant biologic differences of the populations of the various countries, it appears likely that environmental influences of probably dietary nature are responsible.

Comprehensive statistical analyses made by Young and Russell and by Cramer of mortality data in England and Wales, moreover, have brought to light distinct differences in the relative liability of persons engaged in different occupations or belonging to different socioeconomic classes to cancers of the various parts of the alimentary tract. Similar statistical observations were reported by other investigators (Peller; Wassink; Kennaway and Kennaway; Versluys; Hewitt and Brooksbank; Mancuso).

It appears from these data that occupational agents of mainly undetermined nature, as well as habitual factors (tobacco, alcohol), may to some extent account for or contribute to these differences.

Additional evidence suggesting that ingested exogenous agents are responsible for a significant portion of cancers of the alimentary tract may be deduced from the fact that there is a crowding of cancers at sites (physiologic narrows, sphincters, curvatures) of the alimentary tract where the food, chyme, or fecal matter moves slowly and/or remains stationary for prolonged periods (pyloric region of stomach, rectum), i. e., in regions in which the most intense and prolonged exposure to any carcinogens presumably present in the contents of the alimentary tract occurs (Hueper).

Since cancers of the alimentary tract account for a considerable portion of cancer deaths, and in view of the observations cited and considerations offered on the possible role of ingested carcinogens in the production of cancers of this and other organ systems, a thorough investigation of all presently and prospectively used food additives and contaminants for carcinogenic properties seems for these reasons also to be in the public interest.

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Obituaries

JAMES FLEECE RINEHART, M.D.
1901-1955

James F. Rinehart, professor and chairman of the Department of Pathology, the University of California School of Medicine, died of coronary occlusion on Nov. 30, 1955, at the age of 54 years.

After he completed his medical education at the University of California, in 1927, he served an internship at the Alameda County Hospital and then entered the Department of Pathology as assistant, where he remained until 1930. He was appointed Littauer Research Fellow, Harvard Cancer Commission, at the Thorndike Memorial Laboratory, Boston, from 1930 to 1931, after which he returned to the University of California as assistant professor. He was appointed professor and chairman of the Department of Pathology in 1942. He served as a member of the State Board of Public Health from 1942 until the time of his death, acting as vice-chairman of this Board for several years. He was a member of numerous scientific and professional organizations and served as president of the American Society for Experimental Pathology from 1950 to 1951. Through his efforts he developed a capable staff for teaching and research.

His research interests were numerous. Early in his career he worked extensively on hematologic problems. Major contributions were made to our knowledge of rheumatic fever and rheumatoid arthritis by his studies on vitamin C deficiency in these conditions. For many years he was deeply interested in nutritional deficiencies, particularly those resulting from lack of vitamins. His discovery of the relationship between arteriosclerosis and pyriodoxine deficiency in monkeys was one of his outstanding achievements.

In the several years prior to his death he became greatly interested in histochemistry and electron microscopy. These interests resulted in the development of new methods. Significant advances were made in cytophysiology by his studies in electron microscopy.

James Fleece Rinehart, M.D.
1901-1955

Transactions of the American Society for Experimental Pathology

MINUTES OF THE ANNUAL BUSINESS MEETING

Convention Hall, Atlantic City, April 22, 1956

The annual business meeting was called to order at 4:05 p.m. by the President, Dr. Harold L. Stewart. Fifty-three members were present.

1. The minutes of the 1955 meeting at San Francisco were approved as prepared and previously distributed by mail to all members.

2. The Secretary-Treasurer's annual financial report (audited by Drs. Holman and Warner) was approved (Exhibit A—April 1, 1956, in office of the Secretary). The total financial assets (April 1, 1956) are $3,030.60, of which $2,942.49 is in savings account and $88.11 is in checking account; net change (gain) in 1955-1956 was $40.73. The Society annual dues were $5.00 for 1955-1956, of which $4.00 per member was paid to the Federation. Unpaid dues for 1955-1956—nine delinquent members, $76.00; dues in advance $5.00. A.S.E.P. members contributed $216.50 during the past year to N.S.M.R., forwarded through the A.S.E.P. Secretary's office.

3. The Secretary's report follows:

(*a*) The Society has 427 members, 413 active and 14 retired members (the retired members have received *Federation Proceedings* through the A.S.E.P. without payment of dues).

(*b*) The deceased members of this Society reported to the Secretary during 1955-1956 are James F. Rinehart and Joseph Pratt.

By motion duly made, seconded, and carried, "The Secretary is requested to include in the minutes the resolution expressing the regrets and sorrow of members of this Society; the A.S.E.P. honors the memory of these distinguished members."

(*c*) By motion duly made, seconded, and carried, "The resignations of Harry Eagle and J. Karlson were accepted; the requests for change in status from active to retired by M. C. Winternitz and Esmond Long were approved."

(*d*) Dr. Erickson reported that a proposed amendment to the constitution was submitted to the membership as required by Article 8 of the constitution and reads as follows:

> "Article 6, Section 2 (proposed change): "A quorum of the Society for transaction of business shall be 30 active members. . . ." (Second sentence of this section will remain unchanged.)
>
> Motion was duly made, seconded, and approved by unanimous vote.

(*e*) Report on Federation Board, Federation secretaries meeting, and program arrangements (C. C. Erickson): The total number of papers for the 1956 meeting was 121 (18 papers transferred for presentation at other society sessions; 19 papers from other Federation societies scheduled on Pathology program).

By action of Federation Board, the publication of abstracts of "Read by Title" papers will be discontinued.

The problem of rising costs to the Federation and the significant increase in number of abstracts for Part I (abstracts of papers presented at annual meeting),

with suggested "ways and means," was discussed, with a request for comments by members of A.S.E.P. (Raise subscription price; cut length of abstracts; eliminate abstracts; or reduce quality of publication to "throw away" type). The discussion indicated the desire that publication of abstracts of annual meeting be continued! Other suggestions discussed included elimination of publication of annual-member roster, elimination of symposium numbers, and reduction in quality of abstract publication. (Note by Secretary: "Suggestions and expressed views of members were presented to Federation secretaries. Attempt to reduce cost will be made by reducing "editing" and quality of abstract publication.)

The Intersociety Sessions composed of original contributions from members of all six societies were arranged for 1956, for the second trial year, on the five subjects Arteriosclerosis, Blood Clotting and Coagulation, Radiation Biology, Tumors, and Cholinesterase.

Discussion and evaluation of intersociety sessions was requested by the Secretary. Comments and motion expressed commendation and approval of the intersociety meetings and requested the Secretary to continue along the present plan. Muscle, Electron Microscopy, Membranes, and Phase Microscopy were suggested as topics.

The Federation will meet in Chicago in 1957, Philadelphia (or Atlantic City) in 1958.

(*f*) The mail vote made by the members to guide the Council in determining the preference of the Society for journal affiliation resulted in expression of the preference for the A. M. A. ARCHIVES OF PATHOLOGY by a very small margin: preference vote (March 25)—ARCHIVES OF PATHOLOGY, 142; *Laboratory Investigation,* 133; preference vote (April 22)—A. M. A. ARCHIVES OF PATHOLOGY, 155; *Laboratory Investigation,* 152; no preference or unsigned, 6.

4. A report of actions by the Council was made, and the following recommendations were approved:

(*a*) The dues assessment will be $6.00 per member for 1956-1957. Dr. Stewart pointed out that the Society had been operating on a budget of $1 per member per year; that the minimal increase approved was required to pay part-time secretarial help and costs of additional activities of the Society not possible under previous budget.

(*b*) The Society approved the following recommendation in 1955: "The A.S.E.P. would affiliate with or sponsor an established journal, either the A. M. A. ARCHIVES OF PATHOLOGY or *Laboratory Investigation,*" and that "the Council should have the benefit of the vote of the members by written ballot to determine the preference of the Society for one of these two journals."

In accord with the preference of the Society as indicated by written ballot reported by the Secretary, the Council approved the affiliation with the A. M. A. ARCHIVES OF PATHOLOGY and requested the Publications Committee to proceed with arrangements with Dr. Paul Cannon, Editor of the ARCHIVES, to expedite the proposed affiliation.

(*c*) Emeritus or retired members now receiving the *Federation Proceedings* through payment of A.S.E.P. of Federation assessment will continue to do so; this arrangement will be discontinued in the future for those members that request change to retired status; dues for "retired members" will be waived, but emeritus members requesting *Federation Proceedings* will be required to pay the $4 Federation charge.

(*d*) In accordance with elimination of "Read by Title" abstracts by the Federation, acceptance of "Read by Title" papers will be discontinued by this Society.

(*e*) The Council accepted Dr. Robert Wissler's report as A.S.E.P. representative on the Federation Public Relations Committee and approved "in principle the recommendation to establish the office of Public Relations by the Federation" (see report of committees).

Reports of Committees:

(1) Committee on Scientific Man-Power Shortage (Harry P. Smith, Representative of the Federation on the Scientific Man-Power Commission).

The report of this committee of the Federation was abstracted and briefly summarized by Dr. Smith. This report will be published in the *Federation Proceedings* (Exhibit B. Secretary's office).

Dr. Smith, in conclusion, in his memorandum report, suggested: "The Federation can well afford to review policies of graduate education, in light of current problems of recruitment. Our problem is partly one of competing with other disciplines for our share of able students, also in part a matter of maximal utilization of what we have. It is a fair question as to how far we should go in directing the energies of young scientists into research of clinical departments, for these researches center mainly about various categories of disease. The report of the C.N.H. Long committee pointed clearly to dangers involved in expanding categorical research at present. This committee advocated increasing emphasis on basic research. This is consistent with policy of N.S.F. in stressing fundamental research. Are we doing all we can to retain men of promise in our own departments?"

(2) Federation Public Relations Committee.

Dr. Robert Wissler reviewed the recommendations of the Federation Public Relations Committee in 1955 that a "permanent arm" for public relations be established. The Federation in 1955 voted deferment but requested continued exploration of proposal. The aim of this proposal includes education of the public, "year-around basis" of press relations by Federation and biologic sciences rather than press coverage for annual meeting only, dissemination of information to aid in scientific man-power recruitment, etc. The Public Relations Committee has requested the Federation Board to approve in principle the proposal of a permanent public relations program and office, and recommends that a conference be arranged with the Federation Public Relations Committee, governmental agencies, and other science groups, such as A.I.B.R. (discussed by Angevine, H. P. Smith, and Ross McCardle). Dr. Wissler also described Federation plans for a career forum in biology, to be held on Friday, April 20, at University of Pennsylvania for high school science teachers and career counselors in the Philadelphia area.

(3) Report of Dr. J. Walter Wilson (Exhibit C, Secretary's office): Chairman, Committee on Problems of Teaching High School Biology (with Ross McCardle, Hans Schlumberger, and Robert Wissler).

The task of this committee was to consider the proposal of the N.S.T.A. to improve laboratory exercises in teaching high school biology, with the hope of stimulating interest in experimental pathology and increasing the number of students entering the field.

The report recommends, in brief, that "if pathologists are to contribute to the development of better science teaching in our secondary schools two avenues are

open to us: (*a*) to offer courses relating normal and abnormal biology designed primarily for secondary school teachers; (*b*) to prepare a manual of laboratory exercises on normal and abnormal biology to be used in secondary school teaching."

(4) Intersociety Committee for Research Potential in Pathology (Exhibit D, Dr. Stewart's report and articles of incorporation).

Dr. Harold Stewart, A.S.E.P. representative, reported that the Intersociety Committee had been incorporated under the laws of Ohio. The purpose and scope of activities of the Committee were redefined and coincide with those given in the report of the Symposium on Increase in Research Potential in Pathology, published in *Laboratory Investigation* (3:378-450 [Sept.-Oct.] 1954).

The Intersociety Committee has been working with "granting agencies" to develop a pattern of support for research by medical students and research fellows.

Dr. Stewart described the recent establishment of the plan for such support; chairmen of departments can apply for grants to support medical-student Ph.D.'s in research and for support of pathology residents in training for research discussion by Drs. Smith, Castleman, Sprunt, and Wissler.

(5) Progress report, submitted by Dr. Richard Follis, describing the current status of Nutritional Pathology at the Armed Forces Institute of Pathology.

(6) Dr. John Kidd, chairman, Committee on Awards, made the following announcement and recommendation:

"1. That the Society accept with pleasure and gratitude the generous offer of Parke, Davis & Company, tendered through Dr. Bratton and Dr. Hartman, to provide for an annual award for meritorius work in experimental pathology, in the amount of $1,000 per annum, plus necessary travel expenses for the recipient of the award, and a suitable bronze medal.

"2. That the award be called the Parke, Davis Annual Award in Experimental Pathology.

"3. That it be administered by the American Society for Experimental Pathology.

"4. That the award be given for original and outstanding work in experimental pathology to an American investigator who has been selected by a duly constituted Awards Committee (see below), and who shall not have passed his 40th birthday on April 15 of the year in which the award is to be given.

"5. That the Secretary of the Society be instructed to send to all members of the Society, on or about November 15 of each year, an announcement requesting that nominations together with supporting data be sent to the Chairman of the Awards Committee (see below), not later than the following January 10.

"6. That any member of the Society may nominate a candidate.

"7. That the President and Council shall appoint an Awards Committee consisting of a Chairman and 4 Members, all to be members of the Society. The Chairman shall be appointed for a three-year period, and is not to be eligible for further service on the Awards Committee. The Members of the Awards Committee shall be appointed for periods of 1, 2, or 3 years, and may be re-appointed for one additional period, not to exceed 3 years, to serve either as Chairman or as a Member of the Committee.

"8. That the Awards Committee be instructed to make its selection not later than February 15 of each year, and to notify the President and the Secretary of the Society of its selection as soon as possible.

"9. That the Secretary of the Society be instructed to inform the recipient of the award, and invite him to present a paper, based upon the work for which the award was made, at one of the sessions of the Society.

"10. That the Secretary of the Society, with advice from the President and Council when necessary, shall supervise all publicity having to do with the Award."

The motion was duly made, seconded, and unanimously approved to accept the recommendations of the committee and to accept this offer "with enthusiasm, pleasure, and gratitude."

Dr. Stewart summarized the many favorable comments by saying, "I think

the attaining of this award of $1000 for outstanding work in experimental pathology is one of the finest accomplishments of the Society and a noteworthy step by the A.S.E.P." The membership of the committee deserves our word of praise; Dr. Hartman, our appreciation of his successful efforts in negotiating and arranging this significant accomplishment, and Dr. Sweet and Dr. Bratton, our appreciation of their interest and cooperation, resulting in this offer from Parke, Davis & Company.

Discussion and suggestions in reference to awards by Drs. Castleman and Angevine were referred to the Awards Committee for clarification.

Membership and Elections:

The following nominations (66) for membership were approved by the Council, and the candidates were duly elected to membership by action of the Society members:

Charles H. Altshuler, Ellsworth C. Alvord Jr., Stephen B. Andrus, James S. Arnold, Charles Allen Ashley, Serigo A. Bencosme, Kurt Benirschke, Leslie R. Bennett, James M. B. Bloodworth Jr., Jackson J. Clemmons, Jonathen Cohen, John T. Ellis, Patrick J. Fitzgerald, John H. Fodden, Frederick G. Germuth Jr., James W. Goodard, Robert A. Good, Robert T. Habermann, James A. Halsted, William J. Harrington, Charles Harris, Gordon R. Hennigar Jr., Arthur T. Hertig, Samuel P. Hicks, Willard T. Hill, Raymond Hinshaw, Jesus de la Huerga, Robert B. Jennings, E. Elizabeth Jones, Henry S. Kaplan, Roy Korson, Marvin Kuschner, Edwin M. Lerner II, Gerald A. Lo Grippo, Louise S. Lombard, Sarah A. Luse, Donald G. McKay, Guido Majno, Earl J. Mason, John B. Miale, Edwin T. Nishimura, Robert M. O'Neal, Theodore C. Panos, Richmond T. Prehn, Donald A. Rowley, Agens B. Russfield, P. M. Schabel Jr., John A. Schilling, John L. Shapiro, Philippe Shubik, Ira Singer, Leon Sokoloff, Benjamin H. Spargo, Frederic W. Stamler, Mario Stefanini, Frederick Stohlman Jr., Edgar B. Taft, Alan P. Thal, Wilbur A. Thomas, Helene W. Toolan, Gordon E. Vawter, Bernard M. Wagner, Roy L. Walford Jr., Alvar A. Werder, Jean K. Weston, William L. Williams.

The following officers and councilors were elected for the year July 1, 1956, through June 30, 1957.

President,	Frank W. Hartman
Vice-president,	Emory D. Warner
Secretary-treasurer,	Cyrus C. Erickson
Councilors,	William B. Wartman (1955-1957, elected in 1955)
	J. F. A. McManus (1957-1958), incoming member
(Past president)	Harold L. Stewart

Special Committees.

Meritorious Award or Honors:	John Kidd, chairman
	William H. Carnes
	Frank W. Hartman
	Thomas Kinney
	Douglas H. Sprunt
Publications:	William B. Wartman, chairman
	Robert Wissler
	Cyrus C. Erickson
Membership Survey Committee:	Emory Warner, chairman
	Frank J. Dixon
	Charles E. Dunlap
	Willard T. Hill
	Sheldon C. Sommers
	Charles L. Yuile

A.S.E.P. representatives to other organizations.

The Intersociety Committee on Increasing Research Potential in Experimental Pathology: Harold L. Stewart

Division of Medical Sciences, N.R.C.: Russell L. Holman

Council of American Association for Advancement of Science: Charles Randall, Emory Warner
The Eli Lilly Awards Committee (jointly with the Society of American Bacteriologists)
For nominations: Alvin J. Cox Jr.
For awards: William M. Hale
The Committee for Placement Service: Kenneth Brinkhous
The Commission for Biological Stains: J. F. A. McManus
Beaumont House Committee (F.A.S.E.B.): Harold L. Stewart

The meeting was adjourned.

CYRUS C. ERICKSON, M.D.
Secretary-Treasurer
American Society for Experimental Pathology

News and Comment

ANNOUNCEMENTS

American Association of Pathologists and Bacteriologists.—At the last meeting of the American Association of Pathologists and Bacteriologists, held in Cincinnati, on April 26, 27, and 28, the following officers were elected for the ensuing year: president, Granville A. Bennett; vice-president, Sidney Farber; secretary, Edward A. Gall; treasurer, Elbert De Coursey; incoming member of council, John G. Kidd; assistant secretary, Benjamin H. Landing; assistant treasurer, Elson B. Helwig.

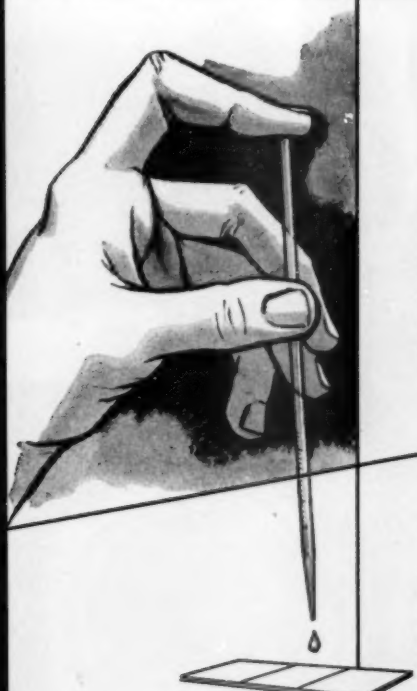
The next meeting of the Association will be held at the Hotel Statler, Washington, D. C., on April 11, 12, and 13, 1957. In addition to the scientific sessions held at this meeting, there will be a symposium entitled "Diseases Caused by Environmental Factors (Dust, Gases, and Other Physical and Chemical Agents)." The referee for this symposium will be Dr. W. C. Hueper, of Washington, D. C.

DEATHS

Dr. John A. Saxton Jr. Dies.—Dr. John A. Saxton Jr., director of laboratories for the St. Louis City Hospital and a member of the staff of the Barnes Hospital, St. Louis, died Feb. 15, of cerebral hemorrhage.

PERSONAL

Morris L. Parker Award to Dr. Israel Davidsohn.—Dr. Israel Davidsohn, head of the Department of Pathology at the Chicago Medical School, received the Dr. Morris L. Parker Award for meritorious research on June 16. The award of $500 was conferred by the faculty in recognition of Dr. Davidsohn's many significant contributions to pathology.



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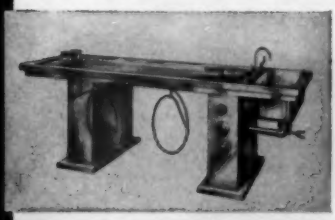

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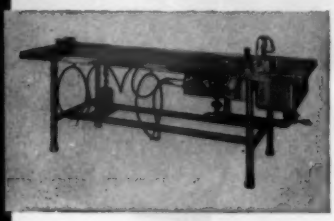

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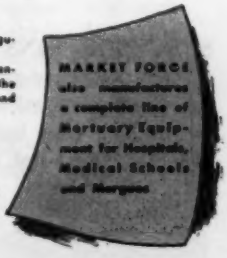

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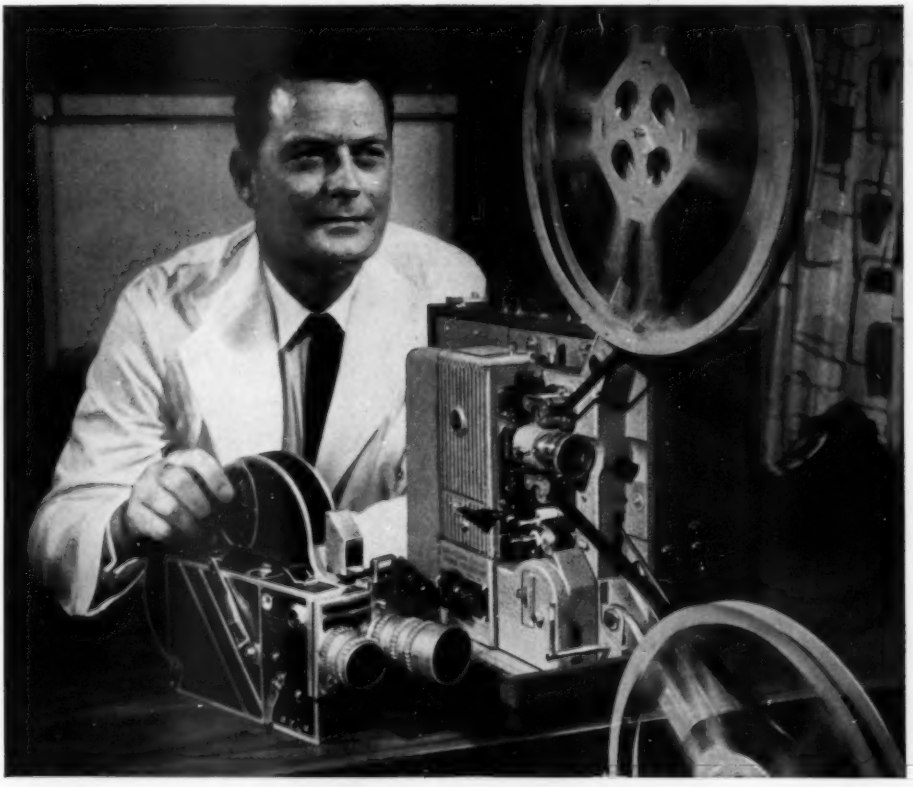

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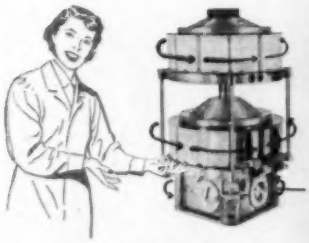

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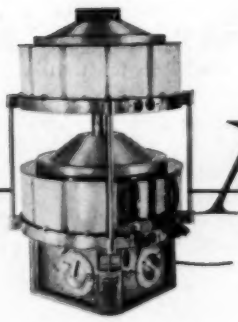

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